2004 PLANT PEST DIAGNOSTICS LABORATORY REPORT

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Cover photo: Seed with net-like seed coat of *Castilleja*, Indian Paint brush, a California native plant. Photo by CDFA PPDB Seed Lab staff.

PLANT PEST DIAGNOSTICS BRANCH
ANNUAL REPORT 2004

Umesh C. Kodira, Branch Chief

Mission:

The primary mission of the Plant Pest Diagnostics Center (PPDC) is to provide timely and accurate plant pest diagnostics in support of the pest prevention system for the California Department of Food and Agriculture (CDFA). The branch also serves as a scientific resource and provides professional expertise to a number of clients including CDFA, the United States Department of Agriculture, other federal and state agencies, County Agricultural Commissioners, the University of California Cooperative Extension, the agriculture industry, and the public. Our scientists, technicians and support staff strive to provide excellence in service and leadership in plant pest diagnostics and biosystematics.

This annual report is a summary of accomplishments of the past year. It provides updates on projects and highlights critical areas of research and new methodology in diagnostics but is by no means inclusive of all work performed at the PPDC.

2004 Sample Workload:

The number of diagnostic samples processed in 2004 at PPDC includes:

Botany	1,008
Entomology	45,000 (estimate)
Nematology	3,874
Seed sciences	6,923
Plant Pathology	109,398 (includes samples from Phytosanitary Quarantine program, seed health testing, and special projects such as Sudden Oak Death, Plum Pox, Nursery Stock Virus Certification, Pierces Disease, and Karnal Bunt.)

These sample numbers are in no way representative of the actual amount of time or labor required to complete any given sample. Nor can sample numbers be compared among the different disciplines (labs) as a measure of workload. Note for example, that the number of Botany or Seed samples does not reflect the number of actual identifications made for a given sample in these labs. It is common for a single plant or seed sample to require multiple identifications of all the material in a sample. Thus a more accurate representation of the true workload for Botany and the Seed Laboratory would be several times these numbers. In a similar way, sample numbers alone do not differentiate between an insect identification that is an immediate recognition and identification from one requiring lengthy study, possibly collaboration with other experts, or even a new published description. Likewise, sample numbers of plant pathology do not

differentiate from those requiring only a simple, quick serological test, from a sample requiring days to weeks of culturing, microscopy, greenhouse testing, etc. in order to arrive at a diagnosis. And, of course, the same line of reasoning is true for Nematology samples as well.

Research:
The scientists at PPDC continue to do research and publish scientific papers as part of the mission of this branch. In the past year, 55 scientific papers were published. A list of scientific publication is included at the end of this report. In addition, numerous formal scientific presentations are given throughout the year by the many of the staff to scientific peers, government agencies, and industry.

Seminar Series

The Plant Pest Diagnostics Center started a seminar series to enable scientists to present research data and discuss on-going research and pest issues of general importance. The focus of the seminar is to share information on any aspect of basic or applied research or diagnostic responsibilities and includes invited speakers from other institutions. Dr. Shaun Winterton, Associate Insect Biosystematist is coordinating the Seminar series.

Staffing Changes:
Mr. Raymond Gill, Program Supervisor, retired from the Department after 40 years of dedicated service to the county and the state. Mr. Terry Seeno, Senior Insect Biosystematist (Supervisor), with 38 years of service to the Department, Dr. Ron Somerby, Senior Insect Biosystematist, with 36 years of service to the Department, and Dr. Alan Hardy, Senior Insect Biosystematist, with 32 years of service to the Department, retired in 2004. It is evident that a lot of years of institutional knowledge are lost with their retirement. We thank them for their distinguished and devoted service to the Department and wish them well in their retirement.

Dr. Gillian Watson, Dr. Rosser Garrison, and Dr. Peter Kerr came on board with the PPDC as Associate Insect Biosystematists during the year. Early this year (January 2005), Dr. Samantha Thomas and Dr. Andrew Cline joined our branch as an Associate Plant Pathologist and as an Associate Insect Biosystematist, respectively. We welcome them to our Branch and look forward to many years of rewarding service in the Department.

NEW SCIENTISTS AT THE PPDB

The year 2004 saw a major turnover in our Insect Biosystematist ranks. With the retirements of Ray Gill, Terry Seeno, Alan Hardy and Ron Somerby, the gaps in our diagnostics expertise appeared staggering. Luckily for us, we had the very good fortune to hire four excellent new Associate Insect Biosystematists to fill their very big shoes. In addition, the retirement of Jim Smith in the Plant Pathology Lab left that lab short-handed to perform general plant disease diagnostics including phytosanitary quarantine samples, since so much time, personnel, and resources had to be diverted to the Sudden Oak Death (SOD) emergency project. But we were very fortunate to be able to hire Samantha Thomas, a very capable and experienced diagnostic pathologist from The Ohio State University diagnostic clinic. The PPDB would like to welcome these new scientists, looking forward to many years to come of close collaboration and a great working environment. We also look forward to years to come of continued interactions with our newest emeriti, whom we hope to see as regularly as before! Following is some background information on our newest hires:

Dr. Andy Cline shares the responsibility for Coleoptera (beetles) with Chuck Bellamy. Andy provided insect identification services, in particular Coleoptera, over the last four years for the State of Louisiana in conjunction with the Louisiana State Arthropod Museum in Baton Rouge. Andy has published research on beetle taxonomy, systematics, natural history and evolution. He also has performed behavioral studies on plant bugs associated with cotton, in particular *Lygus* bugs. Andy's current research focuses on the nitiduloid-lineage of the beetle superfamily Cucujoidea. Ongoing projects include biodiversity surveys in North America, Costa Rica, Bolivia, Panama, and Borneo. A few long-term goals include: producing stable subfamilial and tribal classification systems in Nitidulidae, revising the family Smicripidae, and elucidating patterns of fungal feeding in Coleoptera.

Entomologist Andy Cline

Dr. Peter Kerr is in charge of the Arachnida (especially Acari - mites), Myriapoda (millipedes and centipedes) and all molecular diagnostics in the lab. Peter got his PhD in Entomology at the University of Maryland, College Park, after spending several years collecting insects in South America. His doctoral dissertation was on the evolutionary relationships of the fly family Rhagionidae (snipe flies) and its relatives. Peter originally came to the CDFA as a joint CDFA/UC Davis postdoctoral researcher. In this position, he conducted molecular research on the evolutionary relationships among species of *Anastrepha*, a diverse, economically important genus of fruit flies (including Mexican fruit fly, Caribbean fruit fly, West Indian fruit fly, South American fruit fly, and many others). He also worked on developing population-level molecular markers for pathway analysis of the Mediterranean fruit fly.

Entomologist Peter Kerr

Dr. Gillian Watson is in charge of the Sternorrhyncha (scales and mealybugs, whiteflies, and jumping plant lice) and Thysanoptera (thrips). She did her PhD in Aphid Taxonomy at Imperial College and the Natural History Museum (British Museum), London, UK, before spending five years teaching Biology and Zoology intensive courses at University entrance level. She then moved back to the Natural History Museum to work for CAB International Institute of Entomology (IIE) for 13 years as their taxonomist covering Coccoidea, Aphidoidea and Aleyrodoidea. Gillian's work at IIE entailed identification of pest species (mainly scale insects) sent from all over the world; characterization and description of new pest species; development of identification aids; and delivery of 23 short training courses on the identification of Sternorrhyncha (8 in the UK, one in Australia and 15 on location in 10 developing Asian and African countries, using technology available locally). Gillian is author of an electronic monograph and identification aid on economically important armored scale insects and a training manual on mealybug identification; and co-author of three monographs on the scale insects of the tropical South Pacific region and a book on insects of the Maldive Islands. She has published two book chapters, 14 peer-reviewed papers on aphid, scale insect and whitefly taxonomy, more than 25 datasheets on pest Sternorrhyncha, and has compiled the armored scale insect part of the Fauna Europaea database. In recent years at the Natural History Museum, Gillian has helped administer the Insect Information Service and Department of Entomology external grant funds; and as a Scientific Associate, she has also assisted in curation of the scale insect collection and has provided identification cover of this group

Entomologist Gillian Watson

Dr. Samantha Thomas replaces the recently retired Dr. Jim Smith in the plant pathology laboratory. Samantha works on the Sudden Oak Death Project with Dr. Cheryl Blomquist and staff, while also providing valuable expertise in the area of bacterial plant disease diagnostics. In addition, Samantha brings a new area of expertise to the plant pathology laboratory—namely turf grass diseases—having garnered quite a bit of practical diagnostics experience in this field during her PhD work at The Ohio State University. Samantha was on staff with the Plant Pathology Diagnostic Clinic at Ohio State, working in all areas of diagnostics (fungal, bacterial, viral, abiotic, etc), although she specialized in both classical and molecular methods of bacterial diagnostics. In addition, she also ran the Sudden Oak Death Diagnostics Program for Ohio State, and she set up that clinic's molecular diagnostics laboratory. Already familiar with the National Plant Diagnostics Network as a diagnostician for the Northeast Region, she now brings her expertise to the Western Plant Diagnostic Network, of which the PPDB serves as the laboratory for the Southeast United States and the Pacific Territories.

Plant Pathologist Samantha Thomas

Dr. Rosser Garrison is in charge of the Heteroptera (true bugs), orthopteroids (grasshoppers, walking sticks, etc.), terrestrial mollusks and other miscellaneous smaller orders. Rosser was Senior Biologist/Entomologist for Los Angeles County for the last 20 years where he identified all potentially important agricultural invertebrate pests entering Los Angeles County and provided insect identification services for the general public, nurseries, farmers, and granaries. He has broad experience in the identification of all groups of invertebrate pests and will also serve as an important back up for other taxonomic groups. Rosser has published over 50 research papers including three book chapters and four monographs mostly on his area of expertise, dragonflies, but has also published papers on insect population dynamics, scale insects, and parasitic wasps. He is currently working on a two-volume work with two other authors on a treatise on the dragonfly genera of the New World.

Entomologist Rosser Garrison

Dr Shaun L. Winterton is responsible for Auchenorrhyncha identifications (especially Glassy Wing Sharp Shooter) along with the occasional Neuroptera. Shaun also handles molecular diagnostics for GWSS, including conducting research into developing molecular diagnostic protocols for immature stages for GWSS and related Sharp Shooters. Shaun arrived at PPDB in March 2004 from North Carolina State University where he worked on molecular and morphological systematics of Diptera and Neuroptera, as well as a brief term with USDA-APHIS coordinating Lucid interactive key projects. Originally from Australia, he studied Stiletto-fly (Diptera: Therevidae) systematics for his PhD thesis at the University of Queensland. Shaun has published on a wide variety of topics including, aquatic plant diagnostics and biological control, lacewing taxonomy and phylogenetics, insect morphology, and numerous papers on Therevidae and Scenopinidae (Diptera) from Australia and around the world. Shaun is also coordinating the development of interactive diagnostic keys for PPDB using Lucid 3 software. Already the author of four Lucid keys, two of which are online, he is presently developing online Lucid3 keys to leafhopper genera of North America and Chrysopidae (Neuroptera) of the World.

Entomologist Shaun Winterton

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Evidence for a Case of Allopolyploid Speciation in *Salsola* sect. *Kali* (Russian thistle): Morphometric Component

G.F. Hrusa

Botany Laboratory/Herbarium CDA

Abstract
One hundred and sixty-six individuals of *Salsola tragus* L. (type A) and two un-named Salsola forms (designated as types B and C) were compared using morphometric multivariate methods. Haplotype sequences indicated type C was an allohexaploid derivative of hybridization between tetraploid type A and diploid type B (J. Gaskin USDA, pers. comm.). Multivariate analysis of the morphometric data focused on the intermediacy of type C to type A and type B. Plotted in multivariate space, these data support the hypothesis of hybrid derivation. Type C is most common in the geographic zone intermediate to types A and B, but is also found beyond the intermediate region. Both type C and type B require nomenclatural revision. These nomenclatural proposals and an expanded discussion of hybrid speciation will be published elsewhere.

Introduction
The ongoing homogenization of the world's plant biota that is currently following closely on the heels of human overpopulation has become of world-wide interest, if not concern. It is currently one of the premier topics of weed science, if not botanical, discussion. It has generally been axiomatic that these plant species were spreading in their indigenous genetic form, that is, non-indigenous organisms were the same genotypes as were present in their native regions. Recent evidence indicates this is not the situation. Detailed examples of hybridization between non- native taxa have been provided for the genera *Tragopogon* (Rieseberg and Warner 1987; Soltis and Soltis 1991; Abbott 1992)and *Tamarix* (Gaskin and Schaal 2002) where intermediate hybrid forms, not known or rare in their indigenous regions are the dominant weedy type. In addition, ecologists and systematists have discussed the potential of hybridization between and among native and non-native species to evolve sometimes even more aggressive weedy forms (Gaiser 1951; Wagner 1958; Kruckeberg 1967; Boyle and Holmgren 1968; McLeod 1975; Love and Feigen 1978; Christie and Hall 1979; Warwick, Bain et al. 1989; Andersson 1990; Ellstrand, Whitkus et al. 1996; Ellstrand and Schierenbeck 2000; Allen 2001; Costea, Sanders et al. 2001; Gaskin and Schaal 2003). Although focused on non-native taxa, these papers have largely expanded upon G.L. Stebbins' classic analysis "The Significance of Hybridization for Plant Taxonomy and Evolution"(Stebbins 1969, also see Stebbins 1959). Although both articles consider primarily native taxa, they are directly applicable to polyploidy and hybrid recombination in weeds.

Needless to say, the capacity of weeds to hybridize both within species via ecotypes and among species poses a challenge, first to taxonomists who must identify and place communicative names on these plants, including their parents; to weed scientists who must extrapolate from known behavior to potential behavior; to land managers who must anticipate and detect weedy species in their areas of responsibility; and to weed control

specialists, who must apply the data from taxonomy and weed science to a species with potentially no previously known data regarding behavior or distribution.

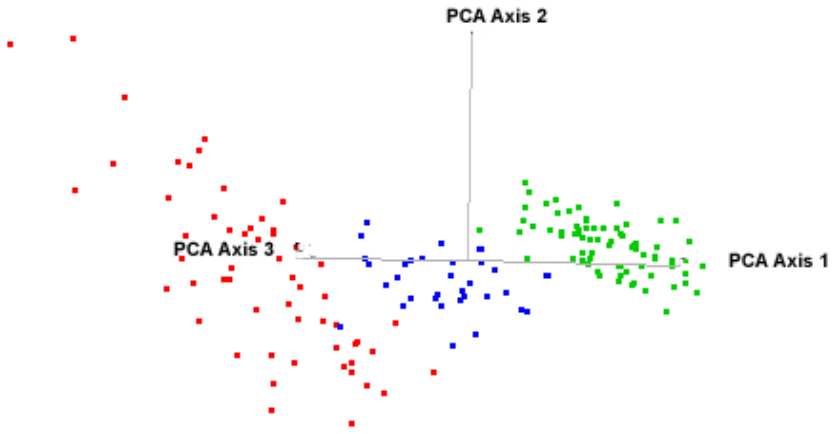
Materials and Methods

Among *Salsola* types A, B, and C, twelve quantitative morphological characteristics defining and describing anther and fruiting wing variation among and within 166 individuals representing most of the known distribution of these plants within California were defined and data acquired. Principal components were extracted and discrimination functions developed that could effectively separate types A, B, and their intermediate type C. Figure 1 graphically displays the location of these individuals on the first three principal components. Figure 2 presents a scaled comparison of the fruiting structures and anthers of the three entities.

Results

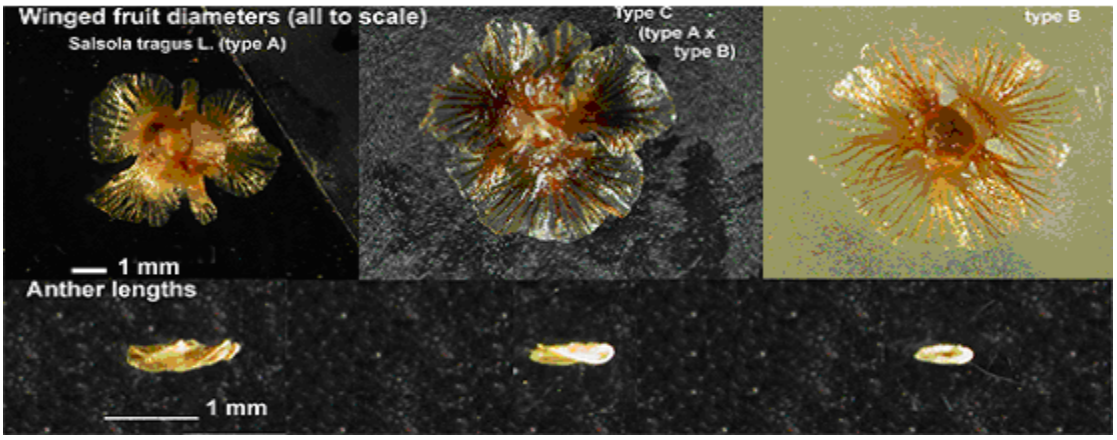
It is clear that fruit wing and anther morphometrics support the phenotypic intermediacy of type C. The clear delimitation of three groups is confounded by their crossing of the three multivariate dimensions plotted below, that is, no one character shows a sequence of transformation from type A through the hybrid type C to type B. This is likely due to the morphological variation being spread across numerous loci. The closest example is overall winged fruit diameter and the length of mature dehiscing anthers (see Hrusa, Figure 2). A multivariate discriminant function to classify individuals into the groupings recognizable in Figure 1 was developed. Using the twelve scored characters this function was effective at classifying all known and suspected individuals of types A, B, or C into one of the three groups.

Figure 1. First three axes of Principal Components Analysis (PCA) of variation among Salsola types A, B, and C.



Red: type A, Salsola tragus s.s., tetraploid (4n) parent
Green: type B, diploid (2n) parent
Blue: type C, hexaploid (6n) derivative of A x B

Figure 2.



Discussion

Currently, Types A and B occur in distinct habitats, and have different reproductive and dispersal strategies (see Hrusa in PPDB Annual Report, 2003). Field work in late 2003 expanded our knowledge of type B distribution and habitat preference, indicating that type C is most common in geographic zones between types A and B which have subtle but distinct habitat preferences. However, there is considerable overlap in distribution between types A and B and the hybrid derivative, type C, is also found beyond the intermediate zone. Type A occurs primarily east of the Coast Range axis, is most prominent in agricultural or other fine-grained soils on roadsides and adjacent to fences, while type B is most common on rocky or gravelly hillsides and rocky road cuts or roadsides west of the Central Valley, It is most common in the South Coast Ranges east of the Santa Lucia Range and on the south coastal plains between Santa Barbara and San Diego. Neither type B nor type C has names available at specific rank. The name *Salsola kali* ssp. *austroafricana* Aellen, based on weedy specimens collected in South Africa, has been proposed for type B there, where it is the dominant type. It also occurs in Australia but its distribution there is not well known. In any case, both type B and type C are distinct species and need nomenclatural application at specific rank. These nomenclatural proposals and an expanded discussion of hybrid speciation will be published in a botanical journal. According to the Rules of Botanical Nomenclature, a name proposed at one rank is not required to be carried to another.

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Annual Report of Seed Laboratory

Sample Workload 2004

Jim Effenberger, Elaine Harris, Don Joley, Deborah Meyer, Paul Peterson, Evelyn Ramos, Marian Stephenson and Connie Weiner

The staff of the Seed Laboratory of the Plant Pest Diagnostics Branch consists of five Seed Botanists, three Laboratory Assistants and additional support from temporary, part-time Scientific Aides supplied by other labs. During 2004, 80% of the workload consisted of seed quality assessment testing and seed/fruit identification, 8% was devoted to laboratory quality assurance (i.e., equipment maintenance and calibration, database management, Q.A. system development, seed herbarium curation) and 12% was devoted to professional enhancement activities (i.e., research, professional meeting attendance, workshop and seminar presentations, professional organization committee work, etc.).

Types of Samples Processed by the Seed Laboratory

The Seed Laboratory routinely handles categories of samples as described below. Table 1 indicates the numbers of samples processed and tests completed during 2004 for each sample type. The percentages of tests completed for each sample type are shown in Figure 1.

- **Quarantine** – Tests on quarantine samples require examination of a minimum of 25,000 seed units from each submitted sample to detect the presence of noxious weed seeds. Quarantine samples are drawn from seed lots moving across state and county lines and are an important part of the pest exclusion, detection and eradication program.

- **Regulatory -**Tests on regulatory label compliance samples include a noxious weed seed examination of a minimum of 25,000 seed units, a purity examination of a minimum of 2,500 seed units, and a germination test of 400 pure crop seed, from each submitted sample to determine label integrity. Laboratory procedures used for these tests are those prescribed in the Federal Seed Act. The noxious weed seed examination is similar to that of a quarantine test. The purity examination determines the physical composition of a seed sample and consists of separation of the pure crop seed kind or kinds (in the case of mixtures of 2 or more species) under consideration from the following contaminants: inert matter, other crop seeds, and weed seeds. The components are reported as percentages based on weight, and all contaminating species are identified. The germination test estimates the percentage of normal seedlings a seed lot can produce. Four hundred seed units are planted on various types of artificial media, and are subjected to various environmental conditions deemed appropriate for the species being tested, in an effort to determine the number of normal seedlings produced under optimum conditions. Laboratory results from the noxious weed seed examination, purity examination, and germination test are compared to the seed lot label; if the results are determined to be out of tolerance with the seed lot label, appropriate action is taken by Nursery and Seed Service. The percentages of the types of regulatory samples released to the Seed Laboratory in 2004 are shown in Figure 2.

- **Service** – Tests on service samples include examinations similar to those described for regulatory tests, as well as specialized tests based on client needs. Service samples are processed on a fee for service basis. The test results are reported directly to the client on formal certificates of analysis and are confidential. These documents are the basis for seed commerce throughout the world. Laboratory procedures used in service testing follow those prescribed in the Federal Seed Act, the Association of Official Seed Analysts Rules for Testing Seed, the International Seed Testing Association Rules for Seed Testing, and the Canadian Methods and Procedures for Testing Seed. Results of these tests may also be used for resolving contractual disputes. The percentages of the types of crops submitted as service samples are shown in Figure 3.

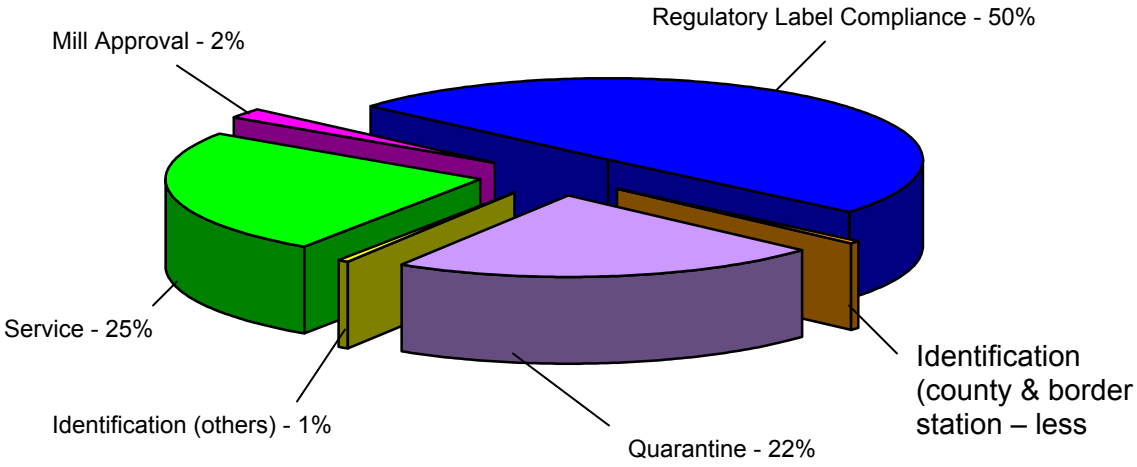
- **Feed Mill Approval** - Feed mill approval tests include the removal, identification, and determination of viability of all weed seed found in processed livestock feed samples. Testing of these samples regulates the certification of feed mills and stops the spread of weed seed throughout the state.

- **Identification** - These samples include identifications of specimens submitted to the laboratory by border stations, counties, other government agencies, commercial seed laboratories, medical doctors, veterinarians, archaeologists, and other researchers. These identifications are not only critical in preventing the spread of hazardous weeds, but are often necessary for expediting importation and exportation of agricultural products, are required as evidence in criminal court cases, and are necessary for medical and veterinary diagnoses of poisoning cases.

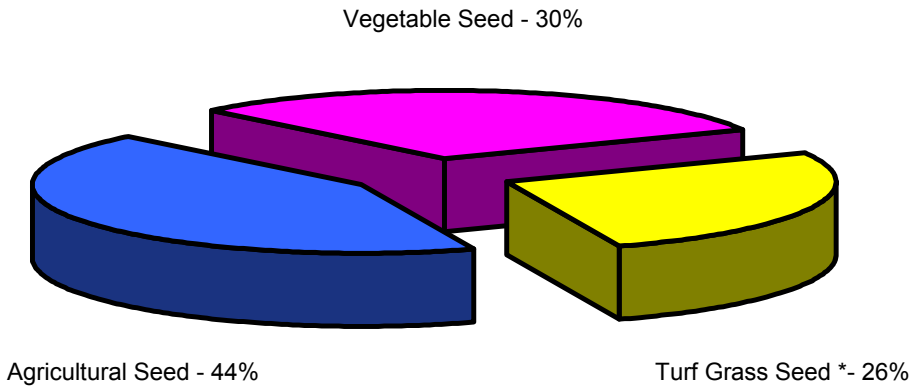
Effenberger et al. Table 1. Total number of samples processed and tests completed by the Seed Laboratory in 2004 for each sample type. Each sample received by the Seed Lab may require more than one test, with the type of test(s) dependent on the sample type.

Type of Sample	# Samples completed	# Tests completed
Quarantine noxious	1540	1540
Identification (county & border station)	30	30
Identification (others)	29	51
Mill Approval	46	126
Service	577	1727
Regulatory label compliance	928	3449
TOTALS	**3150**	**6923**

Effenberger et al. Figure 1. The percentages of tests completed by the Seed Laboratory in 2004 for each sample type. Pie areas represent percentages of the numbers of samples completed, not the time required to complete each type of sample.

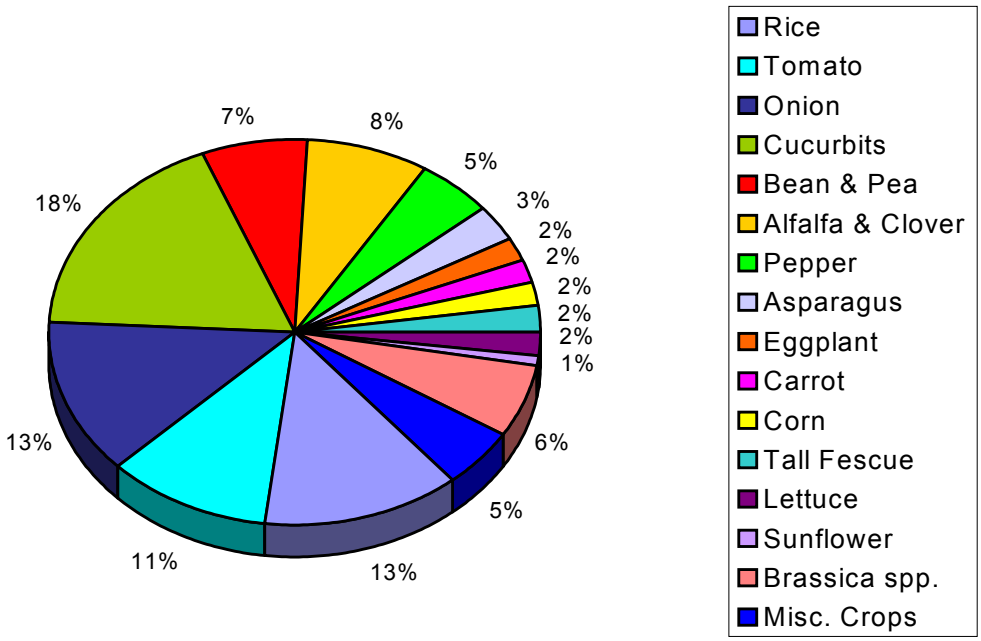


Effenberger et al. Figure 2. Percentages of the generalized crop types of regulatory samples released to the Seed Laboratory in 2004.



58% of turf grass samples contained ryegrass, requiring fluorescence tests.
27% of turf grass samples were mixtures of 2 or more kinds of seeds requiring purity separation and separate germination tests.

Effenberger et al. Figure 3. Percentages of the types of crops submitted as service samples.



Comparison of Purity Testing Methods of Big Bluegrass (*Poa secunda* J. Presl)

Deborah Meyer

The CDFA Seed Laboratory continues to research ways to improve seed quality testing by developing more efficient and less subjective seed testing methods. In 2004, a new method for testing *Puccinellia distans* (weeping alkaligrass) was adopted by the Association of Official Seed Analysts (AOSA) as the official testing method for North America. This method was developed in the CDFA Seed Laboratory and validated by inter-laboratory referee (Meyer and Effenberger, 2004). Research in seed quality testing of *Poa secunda* (big bluegrass) using a similar test method is underway.

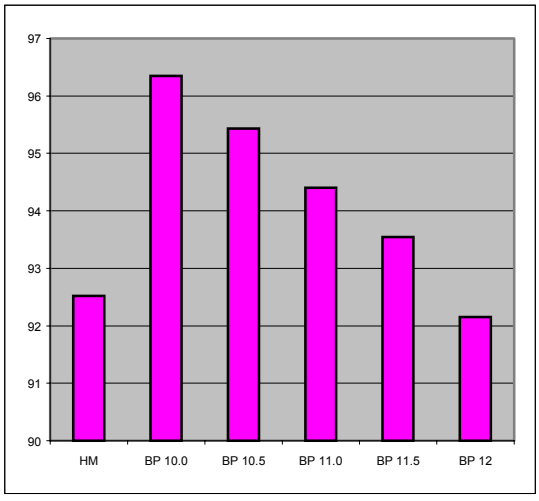
Big bluegrass (*Poa secunda* J. Presl) (Figure 1.) is a small-seeded grass species similar in size to Kentucky bluegrass (*Poa pratensis* L.). This species is used primarily in land reclamation projects. Two purity methods were compared: 1) the hand separation method (HSM), requiring microscopic examination of each seed unit to insure the presence of at least one caryopsis in each seed unit, and 2) the Uniform Blowing Procedure (UBP) similar to the procedure described in the AOSA Rules (AOSA 2004) used for other species of *Poa*, in which empty and underdeveloped florets are removed mechanically via air stream separation in a General Seed Blower. The purpose of the research is to establish a UBP for big bluegrass that provides pure seed percentages similar to those obtained with the currently accepted HSM.

Meyer, Figure 1. Seed units of *Poa secunda* cv. Canby.

Blower calibration established a gate setting of 10.0 for Kentucky bluegrass. Based on the calibration data, five General blower gate settings were selected for the experiment: 10.0, 10.5, 11.0, 11.5 and 12.0.

Preliminary results, based on nine commercial seed lots of three cultivated varieties (Canby, Sandberg and Sherman) of big bluegrass, indicate a UBP between 11.5 and 12.0 will produce similar pure seed percentages as those obtained by the HSM (Figure 2). Once a satisfactory UBP and calibration factor based on the Kentucky bluegrass calibration standard are established a second study will be conducted to provide method validation through inter-laboratory referee.

Meyer, Figure 2. Mean percentages of pure seed across all lots for each blowing point and the hand separation



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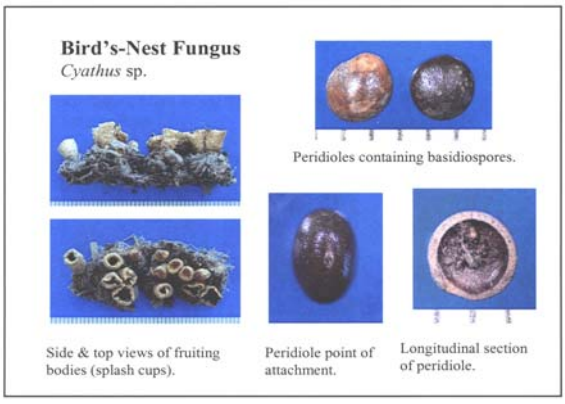
Seed Technologist Training

Jim Effenberger, Elaine Harris, Deborah Meyer, Paul Peterson, Evelyn Ramos, Marian Stephenson and Connie Weiner

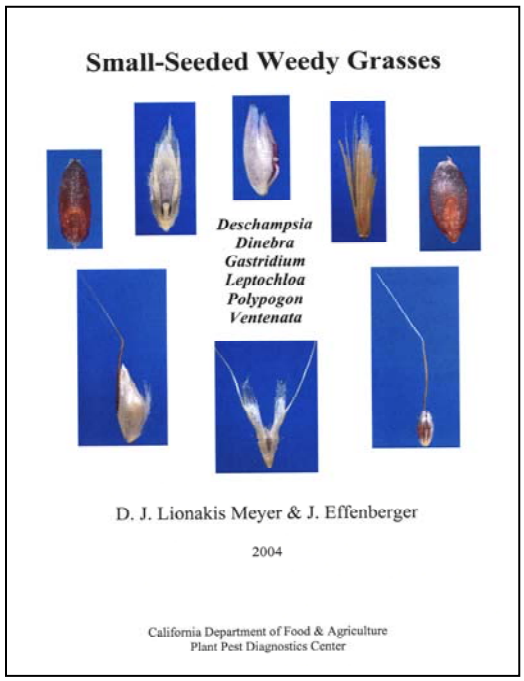
Seeds are the propagules and reservoirs of plant germplasm that farmers rely upon. Scientists involved in seed lot quality assessment must possess an array of skills and knowledge in the areas of purity and germination testing, seed vigor and genetic purity testing. Laboratory analyses serve as the basis for seed trade and thus the exchange of millions of dollars in seed sales globally. Standardization of laboratory test procedures is key to the success of the seed industry. With the goal of promoting standardization among seed testing laboratories, providing training via workshops and supervision of individualized training programs in the field of seed technology is one of the missions of the CDFA Seed Laboratory. Many individuals that have received training from the CDFA Seed Laboratory staff have become Registered Seed Technologists (RSTs) following passage of a nationally administered examination.

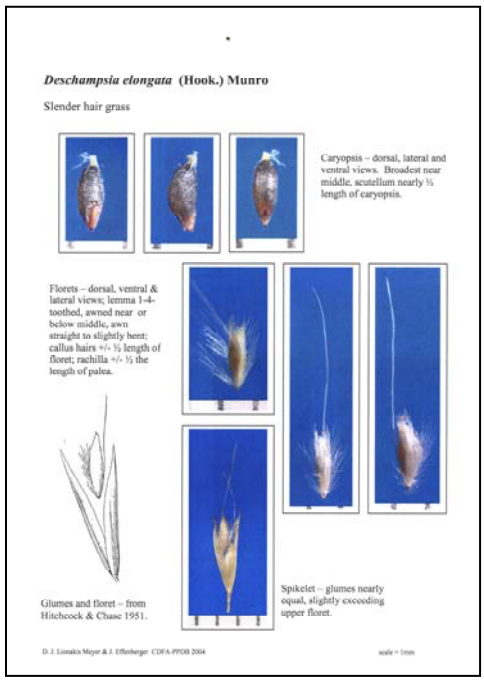
This year Jim Effenberger coordinated the California Seed Analysts and Seed Researchers 2004 Spring Workshop held in conjunction with the California Seed Industry Conference, Woodland, CA and at the CDFA Plant Pest Diagnostics Center, Sacramento, California. The Seed Laboratory technical staff was involved in preparation of hands-on materials for workshop participants to examine. The Seed Laboratory scientific staff made the following presentations:

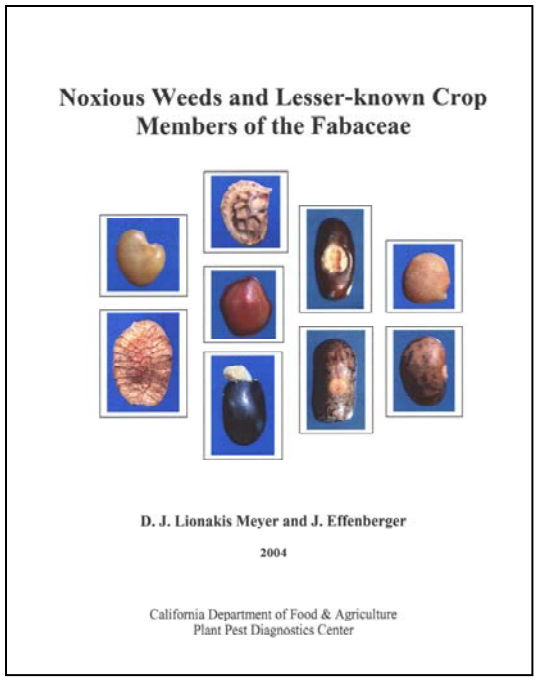
- Paul Peterson, Senior Seed Botanist – Commonly asked questions on germination and seedling evaluation in the following families: Chenopodiaceae, Cucurbitaceae and Solanaceae.
- Senior Seed Botanists Deborah Meyer and Jim Effenberger – Identification of large-seeded Fabaceae (legumes); Identification of noxious weeds and lesser-known crop members of the Fabaceae (legumes); Identification of small-seeded weedy grasses.
- Deborah Meyer – Strange stuff: unusual seed lot contaminants and other things submitted to the CDFA Seed Laboratory for identification.
- Jim Effenberger – Navigating the global seed testing network.
- Tim Tidwell, Senior Plant Pathologist Diagnostician – Identification of sclerotia in seed samples.
- Julie Scher, USDA – New identification tool for federal noxious weed seeds: a Lucid computer-based multi-access key and image database.

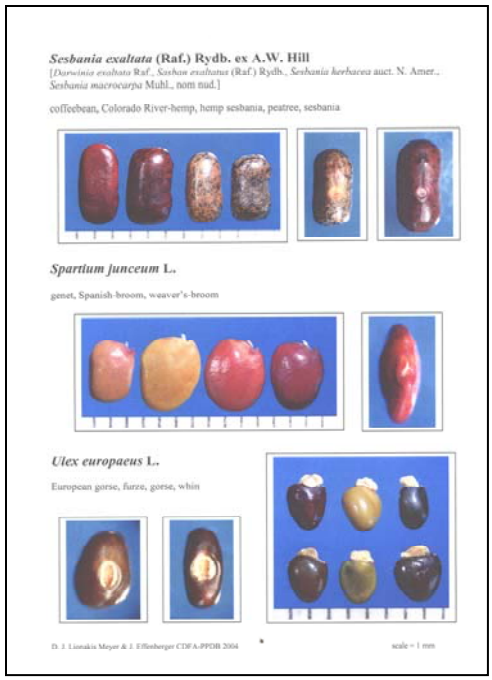


Example of an unusual item received by the Seed Laboratory for identification. In this case the item was a peridiole from a bird's-nest fungus that externally looks similar to a seed. Slide taken from the presentation on *Strange Stuff: Unusual Seed Lot Contaminants And Other Things Submitted To The CDFA Seed Laboratory For Identification.*



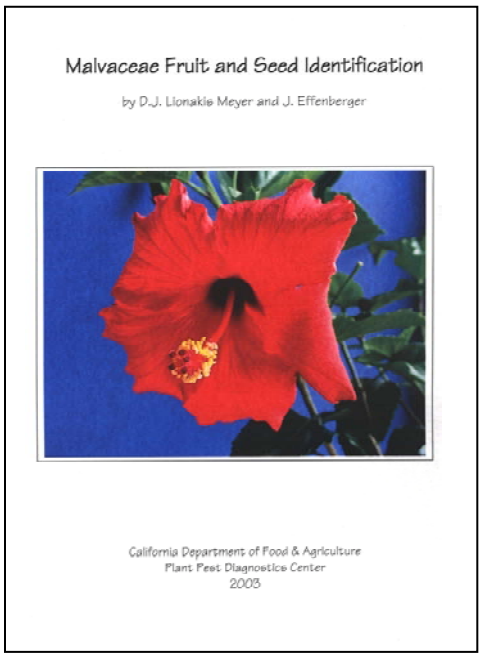


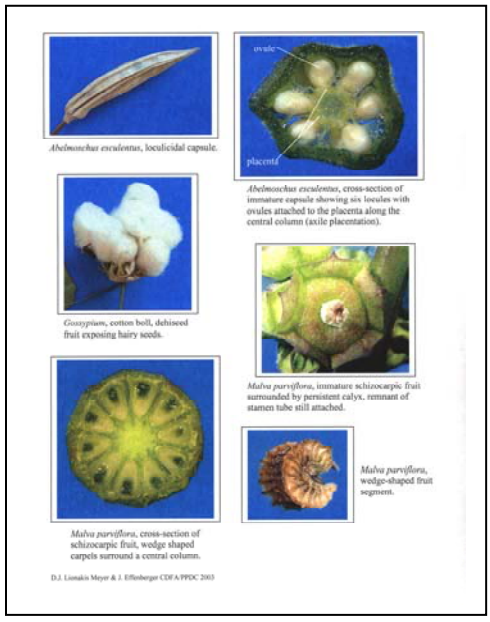


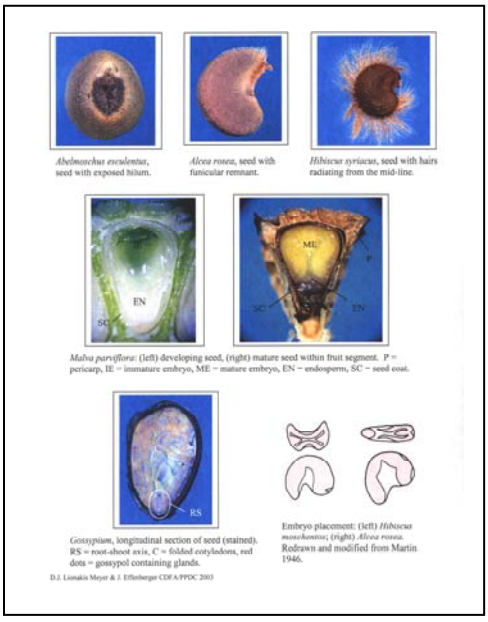


Excerpts from two of the 2004 Seed Workshop seed identification training manuals are provided above. The manual *Small-Seeded Weedy Grasses* provides seed unit descriptions, diagnostic keys, color photographs and illustrations of 17 small-seeded weedy grass species commonly found as contaminants in agricultural situations. *Noxious Weeds and Lesser-known Crop Members of the Fabaceae* contains seed descriptions, color photographs and illustrations of 16 species of minor legume crops and noxious weed species of concern in seed commerce because they are considered noxious weeds either in California or elsewhere in the United States.

At the request of the USDA-APHIS, the CDFA Seed Laboratory provided a compilation of 15 seed identification workshop training manuals developed by the CDFA Seed Laboratory staff. This collection contains 366 color photographs, taken by Deborah Meyer and Jim Effenberger, and 451 illustrations, prepared by Deborah Meyer, representing more than 300 members of seven plant families. The seed identification manuals were distributed to all federal ports for use by USDA agricultural inspectors. Excerpts from one of these training manuals are given below.







The *Malvaceae Fruit and Seed Identification* workshop manual contains general information about the mallow family, 153 color photographs of family, fruit and seed characters for 35 crop and weed species common in seed commerce.

Evaluation of seed quality of a native species: *Atriplex confertifolia* (Torr. & Frémont) S. Watson

Marian Stephenson, Evelyn Ramos, Connie Weiner and Ronny Harley
Photographs courtesy Julia Scher, USDA, APHIS

Atriplex confertifolia is a small shrub native to the arid western U.S. Wildland collections of seed are used to rehabilitate rangeland after fires. Because of extensive genetic differences among populations, revegetation projects are more likely to succeed when seeds from "genetically local sources" are used. The recent conviction, prison sentence and restitution order given a seed supplier for delivery of poor quality seed (of another range species), falsely labeled as to its "ecotype," is testimony to the increasing effort to assure responsibility for seed identity, origin and quality in the native seed industry.

The CDFA Seed Lab cooperated with the Nevada State Seed Lab in testing two lots of *A. confertifolia* fruits. Nevada provided seed collection site inspections and record-keeping for source-identified certified seed. The long term objective of the project was to gather data to develop an official procedure for laboratory testing of the species.

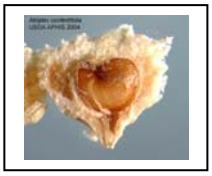
Four-hundred seed units from each sample were tested according to the AOSA procedures for *Atriplex canescens*; after a 7-day oven drying regime; after a 7-day prechill at 10 C; and after hot water treatment.

Emergence of seedlings possessing the essential structures necessary to continue development to become plants was no higher than 2% under any of the conditions.

Ungerminated seed units at the end of the germination test were cut and excised seeds were tested in tetrazolium chloride for viability.* "Empty" or unfertilized fruits comprised 50-72.50% of a sample (Fig.1). Fewer than 50% were "filled," containing seeds that appeared to be mature (Fig. 2).

Figure 1. (left) Ovary and styles revealed by removal of one of two bracts of a *A. confertifolia* "seed unit."

Figure 2. (right) Samples tested had 26.50% - 48.00% seed units containing seeds appearing to be mature.



Stephenson, et al. Table 1. Results of germination, cutting and tetrazolium tests on 2 lots of *A. confertifolia*

Pre-germination Treatment	Sample 1			Sample 2		
	15°C No trmt	15°C Pre-dry	15°C Pre-soak	15°C No trmt	15°C Pre-dry	15°C Pre-soak
% germination	1.00	2.00	0.50	1.00	2.00	1.75
% abnormal seedlings	0.00	0.00	0.00	0.50	0.25	0.25
% ungerminated, TZ viable	23.50	31.25	24.00	36.25	39.50	44.00
% ungerminated, TZ nonviable	3.00	3.25	6.75	9.50	4.50	4.00
% empty or immature	72.50	63.50	68.75	52.75	53.75	50.00

Using x-ray to identify empty seed units could facilitate assessment seed quality of *A. confertifolia.*

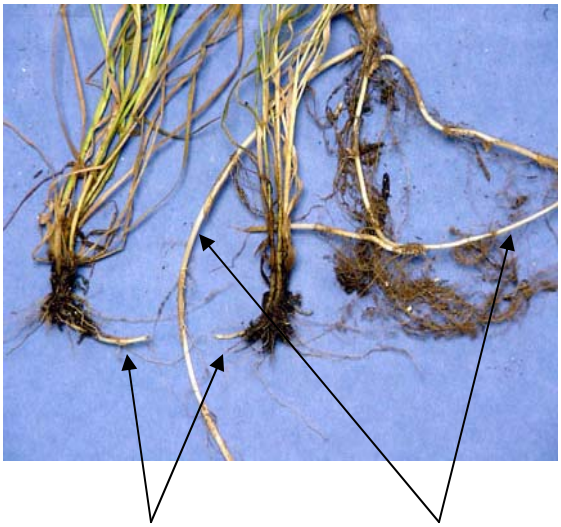
*Seeds treated by pre-chilling germinated at a rate of 1.00% (Sample 1) and 0.75% (Sample 2). Ungerminated seed units were not cut for examination.

Rhizome Evaluation Assists in Newhy Wheatgrass Identification

Jim Effenberger, Evelyn Ramos and Connie Weiner

The recently developed Newhy wheatgrass forms florets that have all the characteristics of one of its parents, quackgrass. The floret is the main structure used when identifying grasses in the seed laboratory and when the characteristics of the floret become debatable other identification characters must be used. Newhy wheatgrass is a cross between *Pseudoroegneria spicata* (Pursh) Á. Löve, bluebunch wheatgrass and *Elytrigia repens* (L.) Desv. ex Nevski, quackgrass. The object of the cross was to create a plant that is vigorous, palatable, drought resistant, and tolerant of high soil salinity. Bluebunch wheatgrass is a perennial with short rhizomes, drought resistance, and is an excellent forage plant that cures well for winter-feed. Quackgrass is a perennial with an extensive rhizome system, an excellent soil binder growing in a variety of soils and a valuable forage grass that is tolerant to high salinity and alkaline conditions. However, too often quackgrass becomes a troublesome weed with its quick-spreading, sharp-pointed rhizomes that have the capability of pushing their way through tubers of potato plants. These rhizomes often extend 3 to 5 feet laterally. The plant is difficult to control because tilling cuts the rhizomes apart and each piece is capable of vegetative propagation. Quackgrass is a B-rated noxious weed pest in California. Breeder descriptions state that Newhy wheatgrass rhizomes average approximately 12 inches in length under field conditions. Our grow-out tests were conducted in a greenhouse at the Meadowview facilities. In our tests, Newhy wheatgrass rhizomes averaged 3 inches in length and quackgrass rhizomes averaged 14 inches in length.

Bluebunch Newhy Quackgrass
Florets



Newhy Wheatgrass Quackgrass
Rhizomes very short Rhizomes

Although the Newhy wheatgrass rhizomes are considerably shorter that the rhizomes of quackgrass, there still appears to be potential for Newhy wheatgrass to become a troublesome weed under certain conditions. The information we obtained from our grow-out tests will be shared with counties within the state that are faced with the question of whether to allow Newhy wheatgrass to be planted in their county.

Flower Seeds Focus of 2004 Book

Marian Stephenson and Deborah Meyer

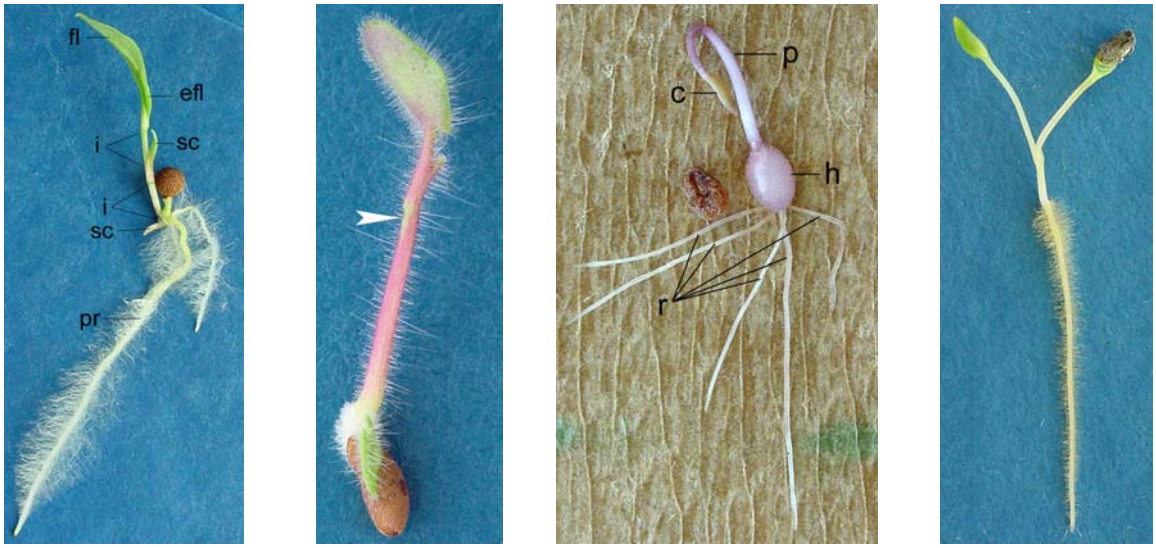
PPDB Senior Seed Botanists Deborah Lionakis Meyer (Supervising) and Marian Stephenson authored chapters in a book, Flower Seeds: Biology and Technology, published by CABI Publishing in 2004.

Although the floral industry is of considerable economic importance in the U.S., Japan and the Netherlands, there previously has been no comprehensive treatment of the subject. This book provides a unique and much-needed resource of information on the biology and technology of flower seeds. International authorities from academia and industry have been brought together to provide a comprehensive reference resource for both practitioners and students of seed science and technology and of ornamental horticulture.

Meyer authored a chapter on seed development and structure of floral crops representing more than 60 plant families. Detailed descriptions of the angiosperm life cycle with emphasis on ovary, ovule, and embryo development, external and internal seed morphology, fruit types, and seed and fruit dispersal are enhanced with 151 color photographs and 128 illustrations by the author.

Alstroemeria, Peruvian lily, flower with ovary highlighted (left); longitudinal section of *Alstroemeria* flower through the inferior ovary (petals and sepals attached above the ovary) exposing the ovules (middle left); *Strelitzia reginae*, bird-of-paradise, seed with orange hair-like aril that attracts birds for seed dispersal (middle right); *Castilleja*, Indian paintbrush, seed with net-like seed coat (right).

Stephenson contributed a chapter on laboratory germination testing of flower seed, reviewing the history of the development of testing protocols by national and international seed testing organizations, comparing existing germination procedures published by the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA) for some 95 species/cultivars and describing normal and abnormal seedling development. The chapter is illustrated with more than 100 color photographs of 24 species, including normally developing seedlings and seedlings with defects.



Alstroemeria, a hypogeal monocot with compact cotyledon and a primary shoot with elongated internodes and scale leaves that develop before foliar leaves (left); *Pelargonium* seedling with defects including only one attached cotyledon, a hypocotyl lesion of undetermined depth and a primary root of undetermined length obscured by the adhering seed coat (middle left); *Cyclamen* forms a globular tuber at the base of the hypocotyl, several seminal roots, and a stout petiole. The cotyledon blade has emerged in this 28-day-old seedling (middle right); *Delphinium* seedlings (except *D*. *cardinale*) have long-petioled cotyledons, short hypocotyls, and tan root hairs (right).

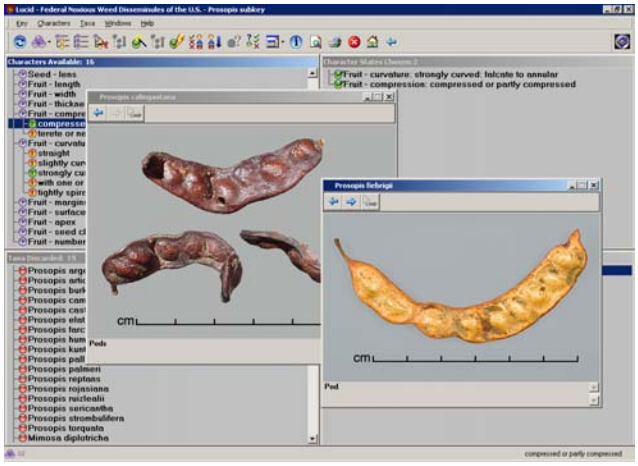
Lucid Identification Key to Noxious Weed Seeds

Julia Scher

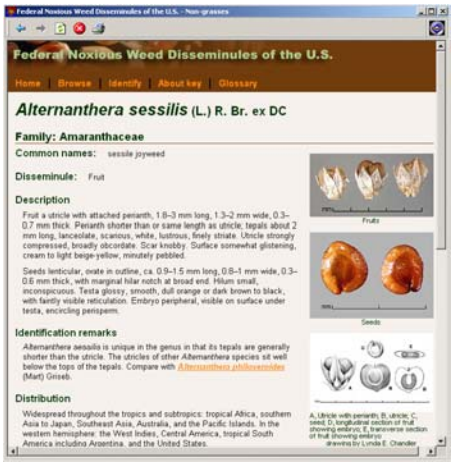
Construction of the Lucid Identification key, "Federal Noxious Weed Disseminules of the U.S." (FNW Disseminules) was completed in late 2004 and is expected to be published as a CD and on the Internet by February 2005. Development of this key began in September, 2002, when a federal-state collaboration was established between the USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine, Center for Plant Health Science and Technology (CPHST), and CDFA, PPDC. It was recognized that resources with which to identify the species on the Federal Noxious Weed List (7 C.F.R. 360) were insufficient and that PPDC, because of its extensive seed collection, library, catalog of images, and the expertise of its seed botanists, was an excellent site for development of the key. An employee was then hired by CPHST to create the key in cooperation with Seed Lab personnel.

Lucid keys are powerful, easy to use, computer-based multi-media identification tools. Lucid keys, which at their core consist of a matrix of morphological data, are fundamentally different from traditional paper-based pathway keys. Identification of a candidate organism can often be reached after only a few keystrokes.

FNW Disseminules covers the fruit and seed propagative units of all the plant taxa (about 100) on the Federal Noxious Weed (FNW) List. The central key matrices in FNW Disseminules are enhanced by 275 HTML files, among them fact sheets for all the FNW taxa, a guide to grass morphology, and an illustrated glossary. Choosing character states is made easier by over 100 original illustrations and explanatory notes linked to each state. The key is supported by over 700 high quality images and drawings of the FNW taxa and an additional 45 similar-looking species.



Images of taxa can be compared side by side by clicking on the taxa names in the key matrix



Fact sheets contain information, digital images, and drawings

FNW Disseminules can be used for identification, as a training tool, as an image gallery, and for reference. FNW Disseminules was envisioned as a tool for APHIS identifiers and Department of Homeland Security officers, and will also likely be welcomed by seed analysts in public and private sectors nationwide.

Entomology Laboratory Staff:

Charles Bellamy
Marc Epstein
Eric Fisher
Stephen Gaimari
Raymond Gill*
Alan Hardy*
Peter Kerr
Andy Cline
Terry Seeno*
Ron Somerby*
John Sorensen
Gillian Watson
Shawn Winterton
Scott Kinnee
Ramona Randolph
Mary-Jean Sawyer
Joanne Virone*
Jenny Chau
Matt Fossum
Randy Plant
Joe Posadas
Ernie Riberal
Jo Viray
Kevin Williams
*Retired 2004

Systematics of the Buprestoidea Leach, 1815 (Coleoptera):
Progress report for 2004

C. L. Bellamy

As detailed in the 2003 annual report, my research continues in several of the same main directions:

1. **The Madagascan Coraebini**
www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/MadCor/intro.html

This project continues following a week-long visit in February to the Museum National d'Histoire Naturelle, Paris sponsored by an Ernst Mayr Grant, Museum of Comparative Zoology, Harvard University. I was able to continue the organization of the vast material held in that collection, borrow additional specimens for specific revisionary projects, photograph most of the primary types, and met with a French collector who owns the remaining portion of the great Madagascan Coraebini collection of A. Peyrieras. I was allowed to borrow a selection of unique specimens which will be described in *Coleoptera Buprestidae of Madagascar and adjacent islands: an Annotated Catalogue*, Fauna de Madagascar, with publication planned for mid-2005.

2. **The Buprestidae of Mexico**
www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/Mexico/index.html

A visit to Sacramento in May by Angélica Corona, PhD student, Faculty of Sciences, Universidad Nacional Autonoma de Mexico, Mexico City focused on her dissertation project which I help supervise as a member of her graduate committee. We were able to resolve several difficulties she had and then visited U.C. Davis and the California Academy of Sciences.

A short field trip in October continued the survey of the Mexican fauna, with visits to the Mexican national collection housed at new facilities of Instituto de Biología, Universidad Nacional Autonoma de Mexico and also with Angélica Corona.

The following publication added 10 new species to the Mexican fauna:

Nelson, G. H. & C. L. Bellamy. 2004. A revision of the genus *Paratyndaris* Fisher, 1919 (Coleoptera: Buprestidae: Polycestinae). *Zootaxa* 683:1-80.

3. **The World Catalogue of Buprestoidea**
www.cdfa.ca.gov/phpps/ppd/Entomology/Coleoptera/Buprestidae/WorldCat/intro.html

The page-formatted catalogue files currently stand at 3124 pages and was essentially completed at the end of 2003. The index is currently being assembled and the publication is planned for 2005. The effort to complete this catalogue has resulted in the following publications:

Bellamy, C. L. 2004a. New replacement names in Buprestidae (Coleoptera). *Folia Heyrovskyana* **11**(3-4) (2003):155-158.
Bellamy, C. L. 2004b. Nomenclatural reversals in Buprestidae (Coleoptera). *The Pan-Pacific Entomologist* **79**(3/4):258-259.

The International Commission of Zoological Nomenclature ruled on two applications submitted in 2002:

Bellamy, C. L. 2002. Case 3193. *Chrysodema* Laporte & Gory, 1835 and *Iridotaenia* Deyrolle, 1864 (Insecta, Coleoptera): proposed conservation of usage by the designation of *C. sonnerati* Laporte & Gory, 1835 as the type species of *Chrysodema*. *Bulletin of Zoological Nomenclature* 59(3):185-187.

Bílý, S. & C. L. Bellamy. 2002. *Cyphosoma* Mannerheim, 1837 (Insecta, Coleoptera): proposed conservation, and *Halecia* Laporte & Gory, 1837 (Insecta, Coleoptera): proposed precedence over *Pristiptera* Dejean, 1833. *Bulletin of Zoological Nomenclature* 59(4):249-252.

ICZN 2004a. Opinion 2076 (Case 3193). *Chrysodema* Laporte & Gory, 1835 and *Iridotaenia* Deyrolle, 1864 (Insecta, Coleoptera): usage conserved by the designation of *C. sonnerati* Laporte & Gory, 1835 as the type species of *Chrysodema*. *Bulletin of Zoological Nomenclature* 61(2):128-129.

ICZN 2004b. Opinion 2083 (Case 3205). *Cyphosoma* Mannerheim, 1837 (Insecta, Coleoptera): conserved. *Bulletin of Zoological Nomenclature* 61(3):188-189.

4. **Miscellaneous Publications**

Bellamy, C. L. 2004. Review of: T. Lander. 2003. Révision du genre *Chrysodema*. *The Coleopterists Bulletin* **58**(1):132.

Bellamy, C. L. 2004. Review of: Gussmann, S. & E. Holm. 2004. *The African Jewel Beetles* (Buprestidae: Julodinae). *The Coleopterists Bulletin* 58(3):428-429.

Westcott, R. L. & C. L. Bellamy. 2004. The rediscovery of *Acmaeodera horni* Fall (Coleoptera: Buprestidae). *The Pan-Pacific Entomologist* **79**(3/4):250-251.

New taxa proposed during 2004:

Agrilus gianfrancoi Bellamy 2004a (**replacement name** for *distinctus* (Cobos, 1967))
Agrilus roswitha Bellamy 2004a (**replacement name** for *rubi* Kaszab, 1940)
Capnodis tenebricosa iranica Bellamy 2004a (**replacement name** for *persica* Obenberger, 1945, preoccupied by *iranica* Bogatchev, 1947)
Capnodis tenebricosa alia Bellamy 2004b (**replacement name** for *iranica* Bellamy, 2004)
Cyphogastra stephensae Bellamy 2004a (**replacement name** for *palliditarsis* Théry, 1923)
Megaloxantha purpurascens endoi Bellamy 2004a (**replacement name** for *ryoi* Endo, 1995)
Paratyndaris (*Waltersia*) Nelson & Bellamy, 2004 **new subgenus**
Paratyndaris costata Nelson & Bellamy, 2004 **new species**
Paratyndaris dozieri Nelson & Bellamy, 2004 **new species**
Paratyndaris mimica Nelson & Bellamy, 2004 **new species**
Paratyndaris paralateralis Nelson & Bellamy, 2004 **new species**
Paratyndaris pulchra Nelson & Bellamy, 2004 **new species**
Paratyndaris similis Nelson & Bellamy, 2004 **new species**
Paratyndaris turbida Nelson & Bellamy, 2004 **new species**
Paratyndaris uniformis Nelson & Bellamy, 2004 **new species**
Paratyndaris verityi Nelson & Bellamy, 2004 **new species**
Paratyndaris westcotti Nelson & Bellamy, 2004 **new species**
Sphenoptera fourie Bellamy 2004a (**replacement name** for *longa* Théry, 1955)
Sphenoptera kalashiani Bellamy 2004a (**replacement name** for *serena* Obenberger, 1926)
Sphenoptera waltersi Bellamy 2004a (**replacement name** for *alia* Bellamy, 1998)
Sphenoptera waynei Bellamy 2004a (**replacement name** for *minuta* Théry, 1941)
Steraspis confusa Bellamy 2004a (**replacement name** for *comitessa* Théry, 1943)
Trachys kurosawai Bellamy 2004a (**replacement name** for *mirabiliis* Kurosawa, 1954)
Trachys sakaliani Bellamy 2004a (**replacement name** for *sempronius* Théry, 1948)

PPDB and The Coleopterists Society

During 2004, the relationship between this lab and The Coleopterists Society continued and yet changed.

Terry Seeno finished his term as Society Treasurer, actually exceeding the normal five year by more than two additional years. Despite the widespread knowledge that the Society needed a new treasurer, no one was in our sites. Thus a special plea was sent by Chuck Bellamy, Society President, to all U.S. members in April. The response was heartening and over the course of April and May, we had narrowed to a short list of three variously qualified, interested candidates. The person we felt best about, Floyd Shockley, a PhD student at the University of Georgia, visited Sacramento in May and was trained by Terry. Terry continues to assist Floyd and our lab continues to maintain the backlog of *The Coleopterists Bulletin* and copies of the first two Special Publications of the Society.

Terry and Chuck attended the Entomological Society of America annual meeting in Salt Lake City, November 14-17, with the Coleopterists Society holding their traditional concurrent meetings. Chuck presided at the Society Executive Council meeting on the Monday morning and the general business meeting on Tuesday evening.

During the Tuesday evening meeting, a special plaque created by President-Elect Mary Liz Jameson was presented by Chuck to Terry. That wording and design of that plaque is reproduced below.

At the end of that meeting Chuck ended his two-year term and became Past-President. President Jameson presented Chuck with a T-shirt which read:

COLEOPTERISTS SOCIETY

With Deep Appreciation To

Terry N. Seeno
Coleopterists Society Treasurer
1997-2004

for the legacy that he has created for The Coleopterists Society
and for the passion that he has shown for the science of coleopterology.
During his tenure as The Coleopterists Society Treasurer, he increased by two-fold The Coleopterists Society endowment, created The Coleopterists Society CD-Rom of all *Coleopterists Bulletin* issues, launched a new Coleopterists Society special publication, wisely invested Coleopterists Society funds for coming generations of coleopterists, "electronified" the society membership, promoted the Society and the *Coleopterists Bulletin* with the BioOne initiative, created and maintained the Society's web page, and advanced The Coleopterists Society to a new and higher zenith.

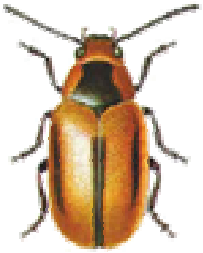
President, Chuck Bellamy

President-Elect, Mary Liz Jameson

Past-President, Bob Anderson

Secretary, Brett Ratcliffe

Treasurer, Floyd Shockley



Editor, Chris Carlton

Salt Lake City, Utah
November 2004

Lepidoptera Lab Report, 2004
Marc E. Epstein

New Pyraloidea in California. In 2004 two introduced species of Pyraloidea in the family Crambidae came to our attention in the Lepidoptera laboratory of the Plant Pest Diagnostic Branch, each new to California. The caterpillars or adults of each were identified by pyraloid specialist M. Alma Solis of the Systematic Entomology Laboratory (USDA). The first, *Lineodes elcodes* Dyar, is a pest on Night Jessamine (*Cestrum nocturnum*). This species has been found in the Santa Barbara area by Guy Tingos and Jerry Davidson. Davidson sent both live and prepared specimens. This species was previously only known from Mexico. The larval and pupal stages were previously unknown and are being described in collaboration with Solis. Davidson and Epstein are documenting this species. The second, *Duponchelia fovealis*, was found on Begonia at a nursery in San Marcus in the San Diego area. This species has been intercepted by APHIS over the last two years from Europe (especially The Netherlands) on a variety of ornamental and vegetable crops (Solis, pers comm.).

Central American moths at IKEA. Be on the lookout this year for imported palm pests. As a specialist in the moth family, Limacodidae, I made the identification of a New World tropical species that is being called "The IKEA Snail Moth" in Europe. This species has been found on Kentia palms (*Howea belmoreana*) being sold at Ikea stores in Denmark, England, Germany and Sweden. The moth is *Acharia* (=*Sibine*) *apicalis* (Dyar), a species that is distributed from Mexico to Costa Rica, reported above 500m in elevation. This species has a caterpillar that is very similar to the North American species known as the Saddleback Caterpillar Moth, *Acharia stimulea* (Clemens). In fact, non-specialists previously misidentified the species as *A. stimulea*, based solely on the caterpillar.

I examined adult specimens of both males and females of the IKEA moth, as well as preserved larvae from all the European countries it has been found in. All match the Mexican and Central American specimens of *A. apicalis* currently on loan at PPDB. The caterpillar specimens match photographs of larvae from which I have identified the reared adults for a study by Janzen and Hallwachs on the caterpillars of Guanacaste Conservation Area in Costa Rica. Unfortunately, it has been difficult to be certain about where the caterpillars originated since the IKEA stores receive plants from centralized nurseries in Europe. While it is possible that the caterpillars remained on the same plant on which they were imported, it is also possible that they may have switched plants. One other Central American species of Limacodidae, *Euclea vericrux* Dyar, was also found on a plant in Germany.

Over 20% of the Costa Rican Limacodid Moths get names. I recently published a paper with Costa Rican collaborator Jorge Corrales describing 25 new Limacodid moths from Costa Rican, a little over a fifth of the 117 species that have been found in the country (see www.zootaxa.com). The larval stages of ten of the species were also described. Color images of both the moths and the caterpillars are included in the publication. Some of the species are quite common in collections, but have remained unnamed because they were confused with other species or are small and inconspicuous.

2004 Auchenorrhyncha Activities

Shaun Winterton

Auchenorrhyncha and Glassy Wing Sharp Shooter Diagnostics: 2004 was a busy year for GWSS with numerous samples identified using morphological, molecular and SEM methods. Research to develop a rapid molecular protocol for Sharp Shooter nymphs is ongoing. A genus level interactive key Auchenorrhyncha families and to genera of North American leafhoppers (Cicadellidae) has been started with over 250 genera put into the key builder. The search for important diagnostics characters is now underway and photographing specimens live and pinned as begun also. Live specimens of thirteen species were photographed for inclusion in the image gallery.

Lucid 3 projects: As mentioned above, work on the interactive key to North American leafhopper genera has begun and will continue throughout 2005. First of the online interactive keys was placed on the PPDB server in December for wider use by the online scientific community. This key formed part of a larger website on Australasian Therevidae (Diptera) (website: http://www.cdfa.ca.gov/phpps/ppd/therevidopen.htm) and was built using Lucid 3 software, allowing the user to run the interactive key on any computer with an internet browser installed. Shaun also published an interactive key to 'Aquarium and Pond Plants of the World' as part of his work with USDA-APHIS. This key is available on CD and online and was developed for quarantine officials to identify aquatic plants in commercial shipments coming into and out of the United States.

Contribution to the "Manual of Central American Diptera" project.
E. Fisher, S. Gaimari and P. Kerr.

Three of the CDFA-PPD Insect Biosystematists, in collaboration with more than 50 dipterists from many parts of the world, are involved with the production of the "Manual of Central American Diptera" (MCAD), being authors for six of the chapters submitted in 2004. When this ambitious, multi-year project is published (hopefully in late 2005), it will then be possible to identify – for the first time – all flies (in 106 families) to the genus-level for this large region of the New World tropics. The production of the MCAD volume is part of the Biodiversity Resources Development Project (BRDP), which is primarily funded by grant money from the World Bank/Global Environmental Facility, plus the countries of Norway and Holland, that was given to the Instituto Nacional de Biodiversidad (INBio) in Costa Rica. (BRDP also has provided funds for similar studies on Coleoptera (beetles), Hymenoptera (wasps) and Fungi.) The INBio facility in Santo Domingo, near the Costa Rican capital city of San Jose, is headquarters for the project, and houses the very large biodiversity collections of animals and plants that are the focus of study. The hundreds of thousands of Costa Rican fly specimens that are preserved at INBio were the original nucleus of the MCAD project; subsequently, all available Diptera specimens from other countries in Central America (Guatemala, Belize, El Salvador, Honduras, Nicaragua, Panama and, in some families, southern Mexico) were incorporated into the study.

When completed, MCAD will have around 113 chapters: one for each fly family, plus introductory ones on morphology, natural history, economic and medical importance, plus family keys for adult and immature flies. Each family chapter will have a similar format, which includes an illustrated key to regional genera, discussions on biology, classification and identification of the family, a synopsis of each genus – which will give details on the current taxonomy and biology of the genus, plus a list of references. Brian Brown (Entomology Curator at the Natural History Museum of Los Angeles County) is editor for the volume. Eric Fisher is author of the chapter on the large family Asilidae (robber flies; Fig. C, shown feeding on a *Urania* moth), which includes some 83 regional genera. These strictly predatory flies are generally considered quite beneficial, as they feed on a great variety of insects (though some species are harmful, as they may also eat honey bees). Stephen Gaimari is author for four chapters in the MCAD, including sole authored chapters on Chamaemyiidae (Fig. E) and Odiniidae (Fig. D), the former being predatory on Sternorrhyncha (aphids, scales, mealybugs, psyllids) and the latter being predators and fungal feeders within tunnels of various wood and twig boring insects. In addition, Steve is first author on two chapters, including Therevidae (stiletto flies; Fig. B) and Lauxaniidae (Fig. F), the former being subterranean predators of various arthropods, and the latter feeding on decaying vegetation. Peter Kerr is the author of the chapter on Rhagionidae (snipe flies; Fig. A). Although some rhagionids are known to be blood-feeders and bothersome to people, the biology of the Neotropical rhagionids is poorly known; most are found in highland, moist habitats where they rest on leaves or tree trunks.

The MCAD project should make a significant contribution to our taxonomic knowledge of the Diptera of a large region of the American tropics. It represents the first comprehensive effort at providing a means of identifying the thousands of different kinds of flies that occur as the tropical neighbors to North American flies. As identification methodology for these insects improves, overall knowledge on Diptera will advance. Flies have a major role in the medical and economic aspects of our lives, and Central America is home to some of California's most important pest fly species.

Figures. Chapters by CDFA/PPD Insect Biosystematists for the Manual of Central American Diptera, as follows: A) Rhagionidae (Chrysopilus sp.) by Peter Kerr; B) Therevidae (Ozodiceromyia nanella) by Stephen Gaimari & Donald Webb; C) Asilidae (Smeryngolaphria numitor) by Eric Fisher; D) Odiniidae (Odinia sp.) by Stephen Gaimari; E) Chamaemyiidae (Chamaemyia polystigma) by Stephen Gaimari; F) Lauxaniidae (Siphonophysa sp.) by Stephen Gaimari & Vera Silva. Photos courtesy of Kevin Holston (B), Stephen Marshall (E,F), Brian Reynolds (A), Alex Wild (D).

RESEARCH ON FLIES (DIPTERA)

Stephen D. Gaimari

Stephen Gaimari's research program has covered many groups of flies and has forged many collaborations, including several foreign scientists. Included in his published work in 2004 are papers with Belgian, Chinese, German, Norwegian, Russian, Swiss and American entomologists. For those published in 2004, these works have covered inventory work (A4, B1), biology and taxonomy of agromyzid leaf miners (A2) and systematic revisionary work for Lauxaniidae (A1, A3), Dolichopodidae (A5) and Empididae (B2). The works finished (in press or submitted in 2004) include studies of biology of predatory flies (C1) and revisionary work on Empididae (C2-5, D1-4).

A. Details were provided in the 2003 CDFA/PPD Annual Report for the following papers that were then in press, and were published in 2004:

1. Gaimari, S.D. 2004. A new genus of Lauxaniidae (Diptera) from New Caldeonia. Zootaxa 449: 1-39. (freely available at http://www.mapress.com/zootaxa/2004f/zt00449.pdf)
2. Gaimari, S.D., L.S. Adler, & S.J. Scheffer. 2004. Plant host affiliation and redescription of *Phytomyza subtenella* Frost (Diptera: Agromyzidae). Proceedings of the Entomological Society of Washington 106 (3): 501-507.
3. Gao, C., D. Yang, & S.D. Gaimari. 2004 (2003). The subgenus *Euhomoneura* Malloch (Diptera: Lauxaniidae) in the Palaearctic Realm. Pan-Pacific Entomologist 79 (3/4): 192-197.
4. Thunes, K.H., et alia (47 authors; S.D. Gaimari, author 34). 2004. The arthropod community of Scots pine (*Pinus sylvestris* L.) canopies in Norway. Entomologica Fennica 15: 65-90.
5. Yang, D., & S.D. Gaimari. 2004 (2003). Discovery of *Systenus* in the Oriental Region, with description of one new species (Diptera: Dolichopodidae). Pan-Pacific Entomologist 79 (3/4): 176-178.

B. The following additional papers were published in 2004, with a brief comment for each:

1. Schacht, W., O. Kurina, B. Merz, & S.D. Gaimari. 2004. Zweiflügler aus Bayern XXIII (Diptera: Lauxaniidae, Chamaemyiidae). Entomofauna, Zeitschrift für Entomologie 25 (3): 41-80.

This paper deals with records of Lauxaniidae (51 species) and Chamaemyiidae (11 species) from the Bavarian region of Germany, including six species that were new records for the country. Additionally, this paper provides a critical updated translation into English of the taxonomic keys to the species of Lauxaniidae of the Palaearctic region from the seminal Russian paper by Shatalkin (2000).

2. Yang, D., S.D. Gaimari, & P. Grootaert. 2004. Review of the species of *Crossopalpus* Bigot (Diptera: Empididae) from China. Transactions of the American Entomological Society 130(2): 169-175.

The genus *Crossopalpus* Bigot belongs to the predatory subfamily Tachydromiinae (Diptera: Empididae). In this paper, we reviewed the species of the genus from China, with two species described as new to science (*C. bisetus* and *C. yunnanensis*) collected in the Xishuangbanna National Nature Reserve, Yunnan Province, and a key to the seven species of the genus from China was presented for the first time.

C. The following papers are *in press*, and will likely be published early in 2005:

1. Noma, T., M.J. Brewer, K.S. Pike, & S.D. Gaimari. 2005. Hymenopteran parasitoids and dipteran predators of *Diuraphis noxia* in the west-central Great Plains of North America: Species records and geographic range. Biocontrol.
2. Yang, D., & S.D. Gaimari. 2005. Review of the species of *Elaphropeza* Macquart (Diptera: Empididae: Tachydromiinae) from the Chinese mainland. Proceedings of the Entomological Society of Washington. 107(1):
3. Yang, D., S.D. Gaimari, & P. Grootaert. 2004. A new genus and species of Tachydromiinae (Diptera: Empididae) from the Oriental Realm. Transactions of the American Entomological Society 130(4).
4. Yang, D., & S.D. Gaimari. 2005. Notes on the species of the genus *Ocydromia* Meigen from China (Diptera: Empididae). Pan-Pacific Entomologist 80(1).
5. Yang, D., S.D. Gaimari, & P. Grootaert. 2005. Review of the species of *Drapetis* Meigen from China (Diptera: Empididae: Tachydromiinae). Journal of the New York Entomological Society 112 (2).

D. The following papers have been *submitted* in 2004, and are currently undergoing review.

1. Yang, D., S.D. Gaimari, & P. Grootaert. 2005. Additions to the fauna of *Drapetis* Meigen (Diptera: Empididae) from China. Proceedings of the Entomological Society of Washington.
2. Yang, D., S.D. Gaimari, & P. Grootaert. 2005. New species of *Elaphropeza* Macquart (Diptera: Empididae) from China. Journal of the New York Entomological Society.
3. Yang, D., S.D. Gaimari, & P. Grootaert. 2005. New species of *Hybos* Meigen from Guangdong Province, South China (Diptera: Empididae). Zootaxa.
4. Yang, D., S.D. Gaimari, & P. Grootaert. 2005. Notes on the species of *Chillcottomyia* from China (Diptera: Empididae). Zootaxa.

CALIFORNIA STATE COLLECTION OF ARTHROPODS: 2004 PROGRESS REPORT.

Charles L. Bellamy & Stephen D. Gaimari

The California State Collection of Arthropods (CSCA) is a scientific resource for the local, federal, and international community for research and identification of various groups of arthropods, especially insects. The collection is maintained by the Entomology Lab of the Plant Pest Diagnostics Branch of the California Department of Food and Agriculture, as an integral feature of the identification services provided to the citizens and business interests of the State, and to our peers and colleagues both nationally and internationally. Two curators (the authors) directly supervise the care, use, growth and development of CSCA, encouraging the use of this collection for research on the taxonomy and systematics of arthropod taxa. The web page for the collection is located at the following URL: http://www.cdfa.ca.gov/phpps/ppd/CSCA.htm.

The total number of prepared specimens exceeds 1.5 million, with more than 30,000 prepared specimens accessioned in 2004. Several holotypes, as yet unpublished, and numerous paratypes were deposited in CSCA in 2004, and the collection is being recognized as an important repository for certain groups of arthropods. The databasing of the collection is in its early stages, with bar-code labels providing unique identifiers for each specimen.

As far as specimen usage, the CSCA issued 22 loans in 2003, representing nearly 10,000 specimens, and more than 25 visitors from the local, national, and international communities have come in to study our collections. Additionally, several client groups have been given tours of the collection. One high school student from Met Sacramento High School has started an internship in the collection.

With the decision to house primary types in the CSCA, we believe that these will need to be available, in perpetuity, for study by the scientific community and thus our need to adequately protect them. While personal examination may always be necessary, we plan to add multiple-view close-up digital images to the CSCA web pages for each type we hold.

The Research Associate program was formally begun with the appointment of four associates:

Steven Chew Kea Foo, Sabah, Malaysia
Scott McCleve, Douglas, Arizona
Natalia von Ellenrieder, Sacramento, California
Kipling W. Will, Berkeley, California

Through these individuals, we encourage the use of the collection, the growth of the collection through their respective donations and allow them to cite their associate status, if necessary, to provide an institutional address for their publication or grants. Several additional scientists have inquired about our program, and several are being considered for this courtesy appointment in 2005. The Research Associates can be found on our website at:
http://www.cdfa.ca.gov/phpps/ppd/Entomology/CSCA/ResAssoc.htm

PPDB ENTOMOLOGISTS: EDITORIAL RESPONSIBILITIES AND SCIENTIFIC SERVICE

Five PPDB insect biosystematists currently serve in an editorial capacity for several scientific journals, and provide other service to professional societies, as follows:

Chuck Bellamy
- Coleoptera Subject Editor: *Zootaxa* (2001-2004)
- Editor-in-Chief*: *The Pan-Pacific Entomologist* (2004 – present)
- English Language Editor: *Folia Heyrovskyana* (2002 – present)
- President: *The Coleopterists Society* (2003 – 2004)
- Past-President: *The Coleopterists Society* (2005 – 2006)

Marc Epstein
- Chairman, Archives and Records Committee, *The Lepidopterists' Society* (2004 – present)
- Lepidoptera Subject Editor: *Pan Pacific Entomologist* (2004 – present)
- Vice President (for North America): *The Lepidopterists' Society* (2004 – present)

Steve Gaimari
- Diptera Subject Editor: *Annals of the Entomological Society of America* (2001 – present); *The Pan-Pacific Entomologist* (2004 – present)
- Editorial Board: *Dipteron, Zeitschrift für Dipterologie* (1999 – present)
- Publications Committee: *The Pan-Pacific Entomologist* (2001 – present)
- Pacific Branch representative, Standing Committee on Systematic Resources: *Entomological Society of America* (2004 – present)

Rosser Garrison
- Odonata Subject Editor: *The Pan Pacific Entomologist* (2004-present)

Shaun Winterton
- Minor Orders Subject Editor: *The Pan Pacific Entomologist* (2004-present)

* Chuck's involvement continues a long history of CDFA scientists holding this position for the journal of the Pacific Coast Entomological Society, including most recently Ron Somerby, and previously Fred Andrews, Bob Dowell, Tom Eichlin, Alan Hardy, Dick Penrose and John Sorensen.

EXOTIC MOLLUSCS INTERCEPTED OR ESTABLISHED IN CALIFORNIA, AND THE IMPACT UPON CALIFORNIA AGRICULTURE

By

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ABSTRACT: Intercepted and introduced exotic mollusks in the state of California are briefly discussed. These include both terrestrial and freshwater genera. The economic and environmental issues for some species are mentioned. The most economically significant established species is the Brown Garden Snail, *Cryptomphalus aspersa*. Molluscicide usages for production of citrus and in the nursery industries are given. The state and local governmental programs for detection and identification of introductions are briefly discussed.

California has one of the major economies in the world. If California was an independent country, its economy would rank fifth or sixth in the world. A major component of this economic power is based upon agriculture, which was valued at over 30 billion dollars in 2002. The State's many rich valleys are geographically isolated by deserts and mountain ranges, which tend to shield it from exotic pests moving by normal dispersal methods.

Since the nineteenth century, the State has attempted to protect sensitive growing areas from accidental or deliberate introduction of exotics by a system of quarantine regulations and inspections. Inspectors are located at all major points of entry into California, including highways, airports and seaports. In addition, County Agricultural Commissioners in most counties conduct routine trapping and inspection activities designed to detect infestations before they reach unmanageable size.

Specimens collected during inspections or trapping are then forwarded to one of several laboratories dedicated to the identification of potential agricultural pests. These laboratories are equipped to identify plant diseases, exotic weeds, detrimental nematodes, and insect, mollusk and vertebrate samples. Many of the most agriculturally significant counties maintain their own identification laboratories, which conduct initial screening of samples. Those samples determined by county identifiers to be potential pests, as well as samples from other agencies and border intercept stations, are then forwarded to the Plant Pest Diagnostics Center in Sacramento for further examination.

The Plant Pest Diagnostics Center of the California Department of Food and Agriculture is the largest laboratory of its type West of Washington D.C., with 25 journeyman specialists, most with PhDs, and an equivalent support staff. The laboratory is housed in a modern building, and includes a large library, reference collections, and electron microscopy and DNA suites. The laboratory processes approximately 30,000 samples a year, not including those identified as a part of an eradication program, which may number in the hundreds of thousands.

In spite of these precautions, there have been successful introductions of marine, fresh water and terrestrial mollusks into California. The introduced marine species have had little or no impact upon agriculture. Major economic loss has resulted from the introduction of terrestrial mollusks, mainly in the families Helicidae and Limacidae, although many other groups have representatives that have become established in California.

The most significant introduced species has been the Brown Garden Snail, a Helicid, *Cryptomphalus aspersa*. The snail has become generally distributed throughout the State, especially in urban and irrigated areas. Major losses from this species have occurred in the cultivation of citrus, and in dooryard and nursery situations. Fully 51% of the metaldehyde, over 17 tons, used in commercial and agriculture situations in California in 2002 was in the production of lemons. An additional 3 tons were used on oranges and grapefruit. Additionally, the fear of accidental introduction of Brown Garden Snail into other areas has prompted governmental agencies in other states and countries to quarantine or reject material from California, unless certified as being from a snail-free facility. This has resulted in additional expense to, especially, the nursery and cut-flower and foliage industries, since such certification requires that the shipment be free of all snail species, including those already widespread in the destination areas. Usage of metaldehyde in nursery situations in 2002 was over five and one-half tons.

Other Helicids established in California include the White Snail, *Theba pisana* (and the Hygromiid *Cerneulla virgata*). There has been an infestation for many decades in the San Diego area, where local population numbers can explode, but they appear to do little damage. Additionally, the Milk Snail, *Otala lactea*, and the Green Snail, *Helix aperta*, have spotty distributions in the southern part of the state, where they mainly occur in dooryard situations. Neither species appears to have much economic impact.

The Cochlicellids, *Cochlicella acuta* and *Prietocella barbara*, have also become established in California, where they can become locally abundant, often in greenhouse situations. Another genus not uncommonly submitted includes what may be several Zonitid species in the genus *Oxychilus*. Populations may occur in dooryard situations in the areas with milder climates, but are more frequently submitted from greenhouse situations. Perhaps the most frequently encountered genus in greenhouse situations are what are probably a number of species in the genus *Succinea.* Major efforts are undertaken to eliminate these snails from commercial greenhouses that export nursery material, in order to retain snail-free certification.

A relatively recent introduction into California was the Hygromiid, *Xerotrichia conspercata*. This species was imported into the San Francisco Bay area on marble, which was then widely distributed throughout the state. After an initial assessment, where several satellite populations were located, a decision was made not to attempt eradication.

Several attempts have been made to biologically control introduced snails, especially the Brown Garden Snail, with little or no success. The Decollate Snail, *Rumina decollata*, a Subulinid, was introduced in a number of locations, most of which proved unsuccessful, although a few populations have survived, most of which appear to be struggling. Further introduction attempts of this and the Spiraxid, *Euglandina rosea*, the Rosy Wolf Snail, have been restricted in California, in the fear that such predator snails could pose a threat to the many native mollusk species, many of which are on the State's endangered or threatened lists.

A number of slugs have become established in California, the most noticeable being the Gray Garden Slug, *Agriolimax reticulatus*, although a number of other Limacid species are present. Common in dooryard situations, it is one of the most abundant introduced mollusk species in California, and can

often be found after dark, wandering about in the mist of homeowner's sprinkler systems. A barefoot nighttime encounter with this species or *Cryptomphalus aspersa* has induced many residents to embark upon eradication wars. Figures for the use of molluscicides by homeowners are not available, but are undoubtedly substantial. Another commonly encountered Limacid is *Lehmannia poirieri*. Not nearly as abundant as *Agriolimax*, it doesn't have the economic impact of the former, but is also targeted by homeowners.

In the cooler, moister portions of the State, several Arionids in the genus *Arion* have become established. Specimen submissions of these species seem to indicate that they are of little concern to either the agricultural industry or homeowners.

The exotic freshwater bivalve*, Corbicula*, has been established in some California waterways for years. The main effect has been the occasional clogging of irrigation pumps and pipes.

California is a major rice growing state, with sales of over $540 million in 2002. In other parts of the world freshwater mollusks have had a major impact upon rice production. The recent discovery of established populations of the freshwater Ampullariids in the genus Pomacea have caused inspectors to focus on surveys to detect populations of Apple Snails. Infestations have been located in a reservoir in the San Diego area, and in ornamental ponds in Northern California and Southern California, and in a tributary to the Salton Sea. The suspicion is that the origin of these populations was from aquariums being dumped in these areas by well meaning hobbyists. Submitted samples have been identified as *Pomacea bridgesii, Pomacea canaliculata*, and *Pomacea haustrum*. The fear is that *Pomacea canaliculata* will move into rice growing areas, with great economic loss. At the present time several counties are aggressively inspecting pet stores, where the snails seem to be widespread and popular.

Within the past several years, infestations of the New Zealand Mud Snail, *Potamopyrgus antipodarum*, have been found in at least two California trout streams. The California Department of Fish and Game has taken the lead in dealing with these populations, with the emphasis being on preventing the further spread into other waterways.

In addition to those mollusks just enumerated, there have been a number of other species established in California, most with negligible impact.

California's quarantine system results in the interception of many exotic species of mollusks that have not as yet become established. Major sources of these interceptions are Hawaii and Florida, most on nursery plants or cut flowers and greens. The most frequently encountered snails are the Pleurodontid, *Zachrysia provisoria*, and the Bradybaenid, *Bradybaenia similaris*, both from Florida. *Bradybaena similaris* is also intercepted from Hawaii, as is the slug *Veronicella*. Other potentially noxious species intercepted have been the Helicids *Cepea nemoralis, Trichia hispida*, and *Helix pomatia*, the Achatinids *Achatina fulica*, the Giant African Snail, and representatives in the genera *Ceciloides, Lamellaxis* and *Opeas*. The slugs *Cystopelta, Pallifera, Meghimatium* and *Philomycus* have also been intercepted.

In addition to the undesirable freshwater mollusks already mentioned, pet store inspections have resulted in the submissions of many common aquarium snails, including those in the genera *Physa, Lymnaea, Helisoma*, and *Planorbula,* most of which pose no pest threat.

The State of California continues to place a high value upon the protection of its agricultural industry. Exclusion of exotic pests and the rapid eradication of incipient populations of pest species are a key component of a program to ensure the quality of food and fiber produced in California. Limiting the spread of established species, and control of local populations costs the agricultural industry millions of

dollars annually. Heavy use of chemical controls has the potential of adverse environmental effects. Pets and native mollusks are adversely affected.

In conclusion, non-native mollusks continue to have an impact upon California, and the risk of additional infestations is increasing. With continuing awareness of these current and potential problems, their impact can be reduced.

Sources: California Department of Pesticide regulation; Summary of County Agricultural Commissioners' reports; Plant Pest Diagnostics Branch records.

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PLANT PATHOLOGY LABORATORY

Jeanenne White

Samples in the Plant Pathology laboratory vary by projects and programs, some of which include partnered efforts with other CDFA branches. 2004 Sample numbers in Plant Pathology break down as follows:

A-rated pathogens identified 13
Q-rated pathogens identified 1288

PLANT PATHOLOGY SPECIAL PROGRAMS	
Seed Health Testing	300
General Diagnostic Samples	5,563
Sudden Oak Death	14,378
Karnal Bunt Project	194
Plum Pox Virus samples	29,465
Pierce's Disease samples	3,900
Nursery Virus Testing	55,598
Total Plant Pathology samples	109,398

SUDDEN OAK DEATH
Cheryl Blomquist

Sudden Oak Death (SOD) caused by *Phytophthora ramorum* is a disease of concern both in the Western United States and, as of last spring, 2004, throughout the rest of the nation as well. SOD-infected Camellias were discovered at a Southern California nursery which ships nursery stock through out the country, and the subsequent discovery of *P. ramorum* in a several California and Oregon nurseries last spring set in motion disease surveys and sampling of virtually every nursery in California and Oregon that ships nursery stock out of state and/or intrastate, as well as in nurseries in states that had received nursery stock from the "positive" nurseries. SOD-positive nursery stock samples resulted in hundreds of additional follow up samples known as "trace backs," i.e. inspections of nurseries from which the SOD-infected nursery stock was believed to have originated, as well as "trace forwards," i.e. samples of nursery stock from inspections of nurseries which had received nursery stock from a nursery deemed "positive" for the presence of the SOD pathogen.

Our laboratory tested approximately 15,000 samples, resulting in over 1,100 confirmations of *P. ramorum* in various nursery plants such as camellias, rhododendrons, Viburnum, as well in California bay laurel and other hosts in infested county wild lands. The sheer size of the work load, the suddenness of the appearance of the infected nurseries, the resultant sudden onslaught of samples, and the continuously adjusting official federal protocols (strategies for sampling and diagnostics had to be continuously adjusted and corrected as more information was learned in the course of the season) made for a very stressful spring and early summer. Couple this with the fact that the nursery stock virus testing project (some 46,000 samples and about 8 employees) and the Plum Pox Virus project (nearly 30,000 samples and about 10 employees) were also going on at the same time, in the same wing of the laboratory as SOD (15,000 samples and about 13 employees) (Blomquist, Figure 1), it all made for a very lively environment!

This spring promises to be just as busy of a SOD season, since SOD-infected nursery stock was just recently found again in one of the Southern California nurseries in which SOD-infected stock was detected last season and compliance sampling and testing has not been completed for all the interstate shipping nurseries. Thus, nursery inspections will proceed again as last year, and thousands of samples will require testing throughout the spring.

The Federal SOD protocol that is followed in the PPDB laboratory is to first visually examine the samples, then prepare the samples for both serological (ELISA) screening tests as well as for agar cultures (Blomquist, Figure 1). For those samples which test positive by the serological test for any *Phytophthora* species, a follow-up molecular test (a "nested PCR test") is conducted on the original sample to confirm the presence of *Phytophthora ramorum* DNA. Generally about 6% of the samples tested by ELISA go on for testing using nested PCR. This test is very labor intensive and time consuming with a single highly-trained technician able to complete a group of 20 samples only every 3 days. In addition, the culture plates of every sample are examined under the microscope by a PPDB staff plant pathologist, and the presence of the fungus in the culture plate constitutes a confirmation of *P. ramorum*.

Blomquist Figure 1. Part of the SOD processing crew, preparing nursery samples for culturing and serological screening.

In addition to nursery stock inspections and nursery sample testing, wild land samples of native tree hosts such as Bay Laurels, tan oaks, and live oaks also continued to come in for diagnosis from the infected counties, over the course of the season. San Francisco and Lake Counties were added to the list of infested counties in 2004 with *P. ramorum* being found on coast live oak in Golden Gate Park and on tan oak and Bay Laurel near the county border just into Lake County.

P. hibernalis was first identified on rhododendron two years ago in Del Norte County. Up until then it was thought to be a pathogen of mainly citrus fruit. In collaboration with Nancy Osterbauer of Oregon Department of Agriculture, we tested the pathogenicity (completed Koch's postulates) of *P. hibernalis* on two rhododendron varieties. This pathogen is important to us because rhododendron infected with *P. hibernalis* can test positive in the nested PCR assay for *P. ramorum*. We are currently writing up the results for publication. (Blomquist, Figure 2).

Blomquist, Figure 2. *Phytophthora hibernalis* infection on Rhododendron.

A Hypothesis about Varietal Resistance and the Kern County PD Epidemic.

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Introduction:

From the mid-nineties to 2001, Glassy-winged Sharpshooter (GWSS) populations grew in the General Beale area of Kern County, until the area-wide treatment of surrounding citrus dramatically reduced the population. It has remained low since. In 2002 the Pierce's Disease (PD) epidemic associated with the GWSS infestation peaked, and then declined dramatically in 2003 and again in 2004. The rate of the increase in PD incidence in the affected vineyards as this epidemic progressed has only been seen in California when GWSS is the vector, and it is consistent with vine-to-vine spread of *Xylella fastidiosa* (*X.f.*). The startling epidemiological discovery in General Beale was that only 2 of the 6 varieties surveyed in the area (Red globe and Crimson Seedless) suffered PD losses. These were the two most susceptible varieties, and all the vineyards planted in these two varieties were affected. The other 4 varieties are considered to be more resistant and they were almost unaffected. There are dramatic examples of Red globe and Flame Seedless vineyards only a few feet apart where the Red globe vineyard was lost (more than 50% PD infected) and the Flame Seedless vineyard had negligible disease incidence. We propose the following hypothesis to explain this varietal difference in the epidemic.

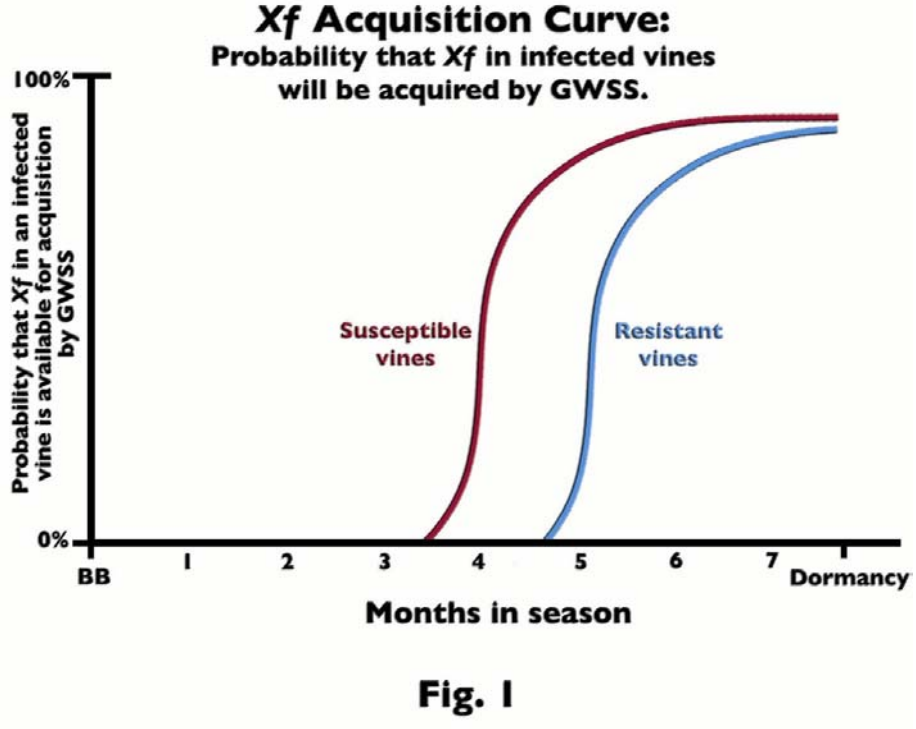
Hypothesis:

We propose that vine-to-vine inoculations were occurring in the vineyards of all 6 varieties. However, in the 2 susceptible varieties many inoculations resulted in infections that survived the subsequent dormant period and progressed to chronic PD. By contrast, almost all of the vine-to-vine inoculations that occurred in the resistant varieties resulted in infections that did not survive the dormant period and the vines were healthy and uninfected the following year.

The phenomenon of over-winter curing of *X.f.* infections has been documented in many areas of California. Early season inoculations result in chronic PD, but later season inoculations die out over the winter. To better understand this phenomenon it helps to visualize that the populations of *X.f.* in grapevines are very dynamic; that the bacteria multiply and spread within the plant during the growing season, but that during dormancy the *X.f.* cells die back and the populations shrink. If during the growing season the *X.f.* populations do not get big enough or do not reach a protected refuge area within the plant, then the bacterial population dies out entirely during dormancy. It is dynamic that results in a climatological limit to the geographic range of *X.f.*, so that in the north where the winter climate is too harsh and the level of plant dormancy is great, any new *X.f.* populations die out entirely no matter how large they may have become during the growing season. Conversely, if the winter climate is very mild then almost any *X.f.* population survives the winter and progresses to PD. Hence there are no *Vitis vinefera* wines in Florida and no PD in New York.

In order for vine-to-vine spread to cause chronic PD two things must happen: 1. *X.f.* in the infected source vine must multiply and spread from it's place of winter refuge in the plant so that bacteria moves into the new growing shoots and grows to populations large enough that GWSS has some probability of acquiring *X.f.* when it feeds. This process takes time and acquisition by GWSS most likely does not begin until sometime in mid-season. 2. When GWSS inoculates a

new vine, it must be early enough in the season so that there is sufficient growing time remaining for the bacterium to multiply and spread to a refuge where it can survive the following dormant period. The probability that either of these two events will occur changes as the season progresses. Graphing the probabilities of these two events produces two curves: a~~n~~ *X.f.* acquisition curve as a function of time (Hill & Hashim, Fig. 1), and an *X.f.* survival curve as a function of time (Hill & Hashim, Fig 2).

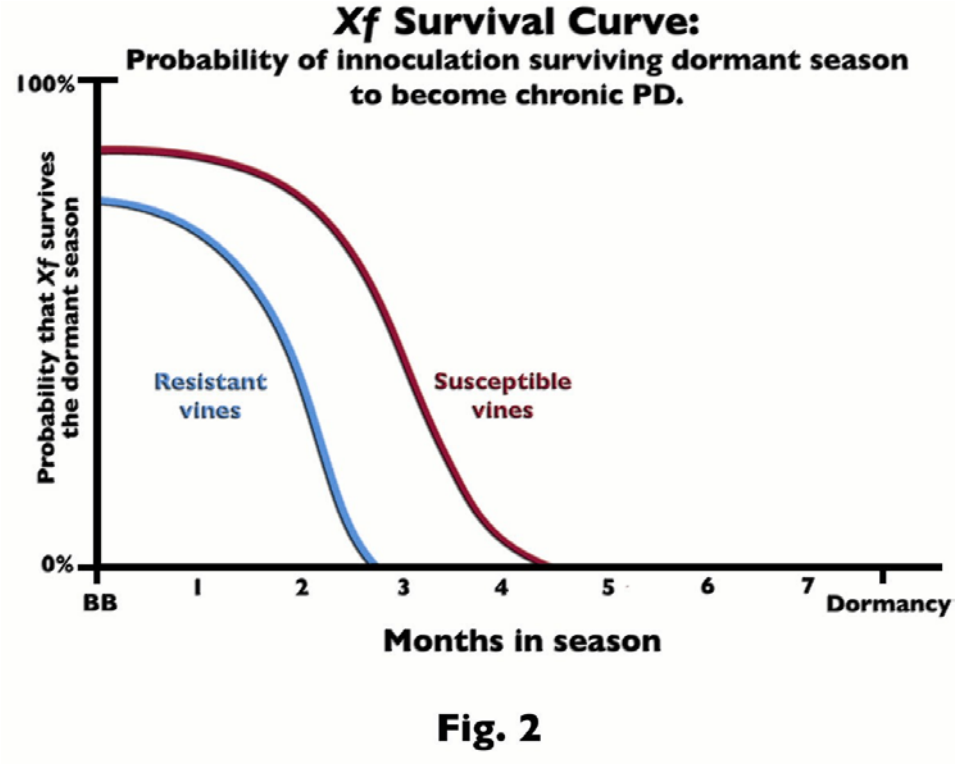


Hill & Hashim Figure 1. *X.f.* Acquisition Curve

Following dormancy, *X.f.* begins to multiply and spread in the plant early in the season around bud break. There is a critical time interval required for bacterial populations to reach new shoot growth so that the bacteria can be acquired by the GWSS feeding there. As evidence for this required interval, laboratory detection of the bacteria in the new growth typically does not begin until at least mid-season. The time interval required until the bacteria are sufficiently present in the shoot for acquisition by GWSS would be a function of the rate of multiplication and spread in the vine. This hypothesis proposes that the rate of multiplication and spread is faster in more susceptible varieties than in resistant varieties. Therefore the *X.f.* acquisition curve for susceptible varieties would begin closer to bud break than the curve for resistant varieties. The result would be distinct curves, one for each variety, represented here by two curves, one for a resistant variety and one for a susceptible variety.

There are other factors in addition to varietal differences that might affect the position of the curve, shifting it either toward or away from bud break. The feeding pattern of the vector is one example. GWSS feeds all along the length of the shoot as well as on petioles. However, other California vectors, such as the blue-green sharpshooter (BGSS) feed primarily near the more succulent distal portion of the shoot and usually on petioles. Since it takes the bacteria longer to reach the distal end, the acquisition curve for BGSS would be shifted to the right of the curve

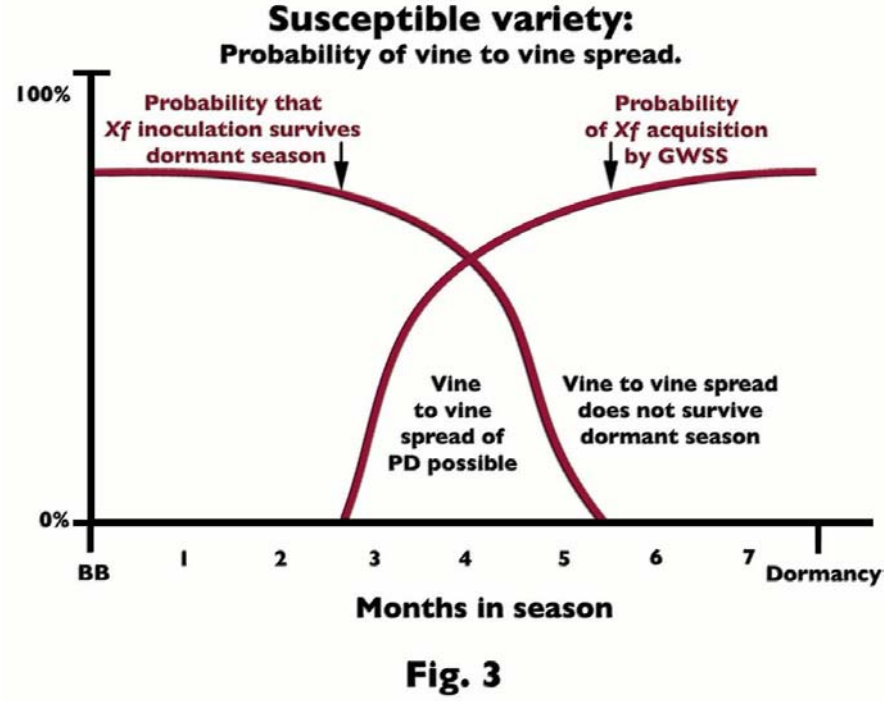
for GWSS. Another factor could be winter climate, a more severe winter climate shifting the curves for the two varieties to the right. Conversely, a more favorable winter climate and growing season would shift the curves to the left.



Hill & Hashim Figure 2. The *X.f.* Survival Curve (Fig. 2)

There is a critical time interval required, between inoculation and dormancy, for bacterial populations to multiply and spread so that enough cells reach and become established in a refuge before the dormancy die off begins. The length of that critical interval between inoculation and dormancy is a function of the rate of multiplication and spread. Because the bacteria multiplies and spreads faster in susceptible varieties than in resistant varieties, the critical interval before dormancy is shorter for susceptible varieties. As with the acquisition curves, the result would be distinct survival curves for different varieties. Any inoculation that occurs after the cut off point would result in an infection that would die off during the winter dormancy, and the vine would be free of infection the following year. This would be an example of vine-to-vine transmission of an *X.f.* infection that does not survive to cause chronic disease, i.e. winter curing.

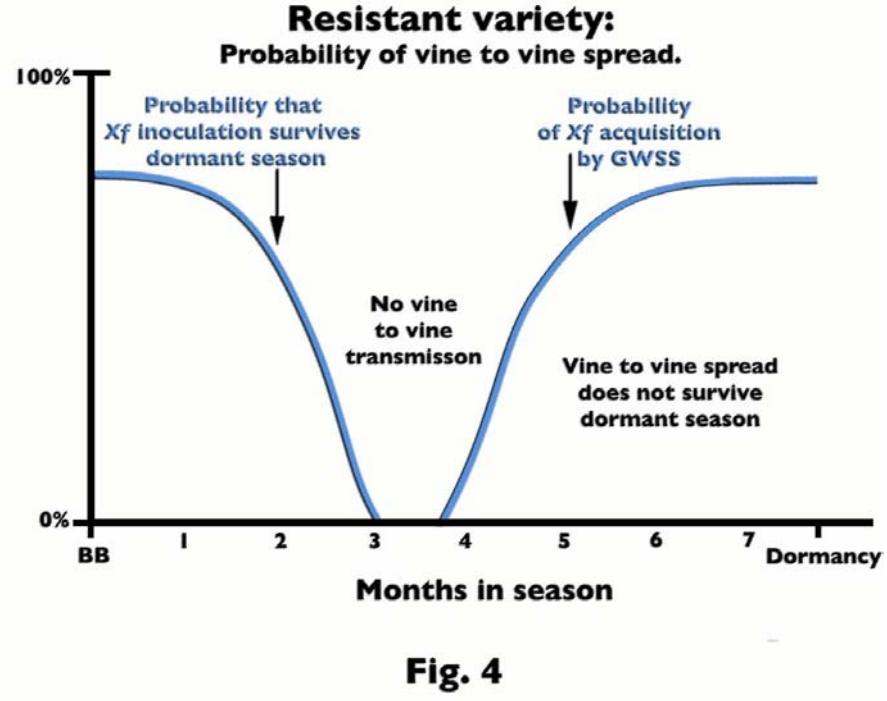
In addition to the varietal influence, there are other factors that could shift the *X.f.* survival curves. A harsh~~er~~ winter climate or a less favorable growing season would shift the curves to the left, closer to bud break. Conversely, a mild~~er~~ winter, or a longer and more favorable growing season would shift the curves left away from bud break.



Hill & Hashim, Figure 3. Combining the Two Curves: the Susceptible Variety.

The *X.f.* acquisition curve and the *X.f.* survival curve overlap in the susceptible variety. This is necessary in order to have vine-to-vine spread that results in chronic PD. The area under the overlapped curves is proportional to the probability that vine-to-vine spread will survive dormancy and result in PD. Of course some infected source vines and GWSS (to feed and transmit) must be present in the first place, or the probability curves would not exist for that vineyard.

There are factors that would affect the amount of overlap of the two curves and therefore the potential for vine-to-vine spread. In comparison to GWSS, a vector such as BGSS that feeds near the distal end of the cane would shift both curves apart. This could eliminate the area of overlap and therefore eliminate any possibility of vine-to-vine spread resulting in chronic disease. A colder winter climate, a shorter growing season, or less favorable growing conditions would have the same effect, i.e. shifting the two curves apart and decreasing the area of overlap. Conversely a milder winter, a longer season, or a more favorable growing climate would shift the curves closer together resulting in a greater area of overlap, a higher probability of vine-to-vine spread, and chronic PD.



Hill & Hashim, Figure 4. Combining the Two Curves: the Resistant Variety.

The *X.f.* acquisition curve and the *X.f.* survival curve do not overlap in the resistant variety. Therefore vine-to-vine spread cannot result in chronic PD. Vine-to-vine inoculation occurs, but it is too late in the season, and such inoculations result in infections that die out during the dormant period. The separation of these two curves illustrates the classic historical situation in California in all varieties and almost all growing areas with other native vectors such as BGSS. Classic transmission of chronic PD in California has been from sources outside the vineyard rather than from vine-to-vine spread.

There are factors that would affect the amount of separation or overlap between the two curves. A longer growing season, or a milder winter would move these two curves closer together, possibly resulting in overlap. If such overlap occurred then vine-to-vine spread could result in chronic PD even in the resistant varieties. If that had been the case in the General Beale area, the varietal effect would have been somewhat masked, because all the varieties would have exhibited varying degrees of vine-to-vine PD spread. Conversely a shorter season or harsher winter would move the curves further apart, insuring that any infections resulting from vine-to-vine spread would not survive the dormant season.

Conclusions:

If this hypothesis is correct, there are a number of possible consequences that could improve PD management and control in areas where GWSS is present.

- The risk to growers of tolerant varieties in most areas of California may be far less than has been previously assumed.
- PD risk assessment, critical to the viticulture industry, would be improved by confirmation of this hypothesis, and by understanding how vine-to-vine spread dynamics vary around California's grape growing areas.
- There is probably a critical window of time somewhere in mid-season (when the *X.f.* survival and the acquisition curves overlap) when chemical vineyard treatments for GWSS would be most effective in protecting susceptible vines from vine-to-vine spread resulting in chronic PD. Treatments earlier and later in the season may be less important than has previously been assumed.
- Economically important losses due to vine-to-vine spread of PD may only happen in susceptible varieties and when large populations of GWSS are involved. Low but persistent populations of GWSS in Kern County do not appear to have resulted in appreciable losses from of vine-to-vine spread. Treatment thresholds and timing may be re-examined for cost-benefit implications.
- Better-targeted and timed chemical treatments could cost less and be more compatible with other IPM programs.
- Late season vineyard surveys and rouging of infected vines is an important and cost effective management tool to minimize vine-to-vine spread.

Because of the potential benefits and implications for better PD management, it is important to experimentally test this hypothesis. We will be proposing experiments over the next two years to test the components of this hypothesis. Our experiments will be designed to demonstrate that both the acquisition and survival curves are different among varieties that vary in susceptibility to PD. We will propose to work in southern San Joaquin Valley. Ideally other researchers might do similar concurrent research designed to clarify the dynamics of vine-to-vine spread in other grape-growing areas with different climates. We would be happy to work collaboratively with other researchers and cooperators on various aspects of this research.

If you would like an electronic copy of this poster in MS Word via email, contact Barry Hill at: bhill@cdfa.ca.gov. or at 916-262-1120.

EPIDEMIOLOGY OF PIERCE'S DISEASE IN KERN COUNTY AND MONITORING AND CONTROL MEASURES FOR PIERCE'S DISEASE

Barry L. Hill

INTRODUCTION

The Glassy-winged sharpshooter (GWSS) arrived in California sometime in the 1980s, and has subsequently served as a vector for *Xylella fastidiosa* (*Xf*), the causal agent of Pierce's disease (PD) and some other diseases in California agricultural and horticultural plants. Before the arrival of GWSS, PD was a chronic problem in many areas of California, but the magnitude of the damage was consistently as low to moderate levels such that no vineyards were lost. In the mid 1990s in the Temecula wine-grape producing area of Riverside County the population on GWSS reached very high numbers and there was an associated epidemic of PD. Almost half of the vineyards in that area were lost. This was the first time in 100 years of grape production, since1890, that entire vineyards were lost to Pierce's disease. About 4 years later, in the General Beale area of Kern county the population of GWSS again rose to very high numbers and another associated PD epidemic occurred in which several vineyards were lost. For the last three years this laboratory has been assessing the epidemiology of the Kern county PD epidemic. What was found has led to a hypothesis about the mechanism of PD transmission leading to disease and loss of grapevines. This hypothesis has numerous implications for disease control and risk assessment for the grape producing industry.

The cooperative area-wide pest management project for the control of GWSS has defined 7 distinct grape growing areas in Kern County. The PD epidemic that peaked in 2002 only affected two of these, the General Beale and the adjacent Northern area. These were also the only areas where the populations of GWSS exploded in 2000 and 2001 to extremely high populations not seen elsewhere in the county. Insect control measures begun in winter 2001-2002 brought the GWSS populations down dramatically. During this time the population dynamics and control methods for controlling GWSS were studied extensively with effective results. However our understanding of how to control the disease and the epidemiology of PD when the causal bacterium is transmitted by GWSS had been based on only limited actual field data. This project began in 2002 as a multi year assessment to obtain extensive data about the incidence and control of the disease, information that would compliment the insect information to enable understanding of the dynamics of the epidemic and methods to control other potential outbreaks. A total of 216 vineyards with 4060 acres and 2,015,698 vines were surveyed, about 4.6% of the vineyard acres in Kern County.

Because the other five viticulture areas of Kern County did not yet have such high numbers of GWSS, it was thought that disease and insect data from those would provide baseline information in the event that another epidemic such as the General Beale and Northern outbreak might occur, and such an epidemic could be studied from the beginning. Among the other 5 viticulture areas, 4 (Central, South A, South B, and West) have had low numbers of GWSS present since sometime before 2000, and GWSS was discovered in the 5th (Hwy 65-Delano) after 2000. Thus this extensive project to monitor the PD disease incidence in these areas was intended to provide both an understanding of the effect of low populations of GWSS on the incidence of PD, as well as a complete epidemic profile over time if another one should occur in this county.

OBJECTIVES

- Evaluate the importance of epidemiological factors such as GWSS population size, vine age, cultivar susceptibility, control practices, and GWSS control treatments in vineyards and nearby GWSS hosts or habitat.
- Make all the epidemiological data obtained available in a commonly acceptable GIS format for analysis by other qualified researchers and epidemiologists.
- Develop PD monitoring and management techniques and strategies for use by growers to reduce risk and damage. Update and provide educational materials to assist vineyard managers, pest control advisors, other researchers and government agencies involved in advising growers in the area-wide pest management of the GWSS project.

RESULTS AND CONCLUSIONS

Vineyards were monitored by visually inspecting each vine for PD symptoms, and by collecting and testing (by ELISA) samples from symptomatic vines. The results in the General Beale area indicate that the dramatic decrease in the number of infected vines that began in 2003 continued in 2004. From 2002 to 2003 the number of infected vines decreased by 85%, and from 2003 to 2004 the decrease was an additional 68%. Following the survey of these vineyards in 2001 and 2002 the vines found to have confirmed *Xf* infections were removed. The continued decline of *Xf* infection in this area demonstrates that effective PD control can be obtained with a combination of GWSS control, monitoring for infected vines, and removal of infected vines. These projects have demonstrated that vineyard disease monitoring and vine removal is cost effective.

Throughout the county as part of this project vines found to be infected with *Xf* were removed at the end of that season. As a result the surveys in 2003 and 2004 are identifying vines that are newly infected. The rate of infection in all areas of Kern County outside the General Beale and Northern areas is very low, an overall rate throughout the county of less than one new infection per 10,000 vines. By contrast in the General Beale area some of the vineyards developed very high levels of disease within a 2 to 3 year period, peaking in 2002. Several vineyards were entirely lost.

Before the arrival of GWSS, primary spread of *Xf* from sources outside the vineyard accounted for most or all of the PD in California. The rates of new infections in Kern County may be the result of both primary spread and secondary spread that is vine to vine spread. The low rates of new infections outside the epidemic area is consistent with primary spread, but the rapid rates of infection in many vineyards within the General Beale area is consistent with secondary, vine to vine spread.

Perhaps the most startling epidemiological discovery of this project so far was that in 2002, 99% of the PD infected vines in the General Beale area were in Red globe and Crimson vineyards, the 2 most susceptible of the 6 varieties surveyed. The following year, 2003, these same vineyards accounted for 97% of the diseased vines. These two varieties comprised only 18% of the acreage surveyed in the General Beale area. There were dramatic instances where Red globe and Flame Seedless were growing in adjacent vineyards, and the susceptible Red globe vineyards were heavily impacted or totally lost, whereas the more tolerant Flame Seedless vines growing just a few feet away were almost unaffected. The rate of infection in vineyards in General Beale of varieties other than Red Globe and Crimson in any of the three years was less

than 14 infected vines out of 337,693 vines surveyed. In the worst epidemic area in Kern County the infection rate in varieties other than Red globe and Crimson was essentially negligible. The Crimson loss in the General Beale area involved only one vineyard, and these vines were less than three years old. Younger vines are more susceptible to PD than older vines, and it is possible that the losses in the Crimson vineyard were primarily related to their more vulnerable age, rather than a varietal susceptibility. Older Crimson vines may not have been so heavily impacted.

We have developed a new hypothesis that would explain what might be causing this varietal difference. It is based on the timing of when in the season GWSS can acquire *Xf*, when in the season GWSS transmits *Xf* to new vines, and the phenomenon of over-winter curing of *Xf* infections. Over-winter curing of PD has been demonstrated to occur in many areas of California, including the San Joaquin Valley. Populations of *Xf* in grapevines are reduced during the winter dormant season. It has been experimentally demonstrated that if a vine is infected early in the season, the bacterium has enough time left in the growing season to multiply to high enough population levels and spread into areas of the vine where some of the bacterial cells find a refuge and can survive the winter dormancy. The vine then becomes chronically infected and usually eventually dies. Conversely, if a vine becomes infected later in the season, all the bacteria in the vine die over the winter, and the vine is free of disease the following year (1). Also pruning may play some role in over-winter curing. Vines that are inoculated late in the season when there is insufficient time for bacteria to move beyond the inoculated cane would, of course, lose the infection when that cane is pruned. However the bacteria in an un-pruned cane may die over-winter anyway.

Our new hypothesis is predicated on the finding that *Xf* multiplies and spreads faster within a susceptible plant than it does in a more tolerant plant (3). It would reasonably follow that the bacterium would also multiply and spread more rapidly in the more susceptible grapevine varieties of Red globe or Crimson than it would in the more tolerant varieties such as Flame Seedless or Thompson. The <u>first part</u> of our hypothesis is about when in the season a grapevine must become inoculated in order for the bacterium to survive the first winter dormancy in the plant thereby progressing to chronic Pierce's disease. We hypothesize that the tolerant varieties have to become infected with *Xf* earlier in the season than susceptible varieties in order for the bacterium to have enough time left in the growing season to multiply and spread sufficiently in the vine to be able to survive the winter dormancy period. In general it has been demonstrated that vines must be inoculated before some critical time in the season if the bacterium is to survive the winter (1). However the existence of differences among varieties regarding that critical necessary time of inoculation has not yet been experimentally demonstrated.

The second part of our hypothesis is about when in the growing season the bacterial cells, having over-wintered in a previously infected plant, multiply and spread from their winter refuge into the new growth and achieve population numbers great enough to be efficiently acquired by an insect vector, in this case GWSS. This growth and movement of the bacterium following winter dormancy has to happen before vine to vine spread can begin to occur. It is not possible to detect *Xf* in the new growth of an infected plant until sometime about mid-season, and it ha been demonstrated that the bacterium must multiply to relatively high (easily detectable population sizes) before acquisition becomes efficient (4). Because it multiplies and spreads faster, we hypothesize that bacteria become available for acquisition in an infected grapevine of a susceptible variety earlier in the season than in a vine of a tolerant variety.

Putting these two parts of the hypothesis together can explain why the varietal differences in disease rate were observed. In the most susceptible varieties inoculations occurring later in the growing season can result in infections that survive the winter to become chronic. Because of the faster bacterial multiplication and spread there is still enough time in the growing season to reach a threshold for survival. At the same time, the bacteria multiply in previously infected vines fast enough to become available for acquisition by GWSS earlier in the season. The timing of these two processes results in an overlap that is a window of opportunity when GWSS can acquire *Xf* from an infected vine, transmit the acquired bacteria to a new vine, and the new infection has enough time to progress to chronic infection and disease. That window of time would close during the season, but vine to vine transmissions would still be occurring. However those later season transmissions, after the window of opportunity has ended, would be cured over the winter. So vine to vine transmission occurring within the window would become chronic, and vine to vine transmission occurring after the window would be winter-cured.

Conversely in the tolerant varieties infections must occur earlier in the season in order to have enough time, at the slower rate of multiplication and spread, to progress to chronic disease. At the same time bacteria from previously infected vines also multiply and spread slowly and do not become available for vector acquisition until later in the season. The result is that there is no overlap, no window of opportunity where GWSS can acquire *Xf* from an infected vine, transmit to a new vine, and have the newly infected vine progress to chronic disease. In this case all of the vine to vine transmissions occur too late in the season, and the result is that all the vine to vine infections are cured over the winter.

One question is why do epidemics that are vectored by GWSS result in vine to vine disease spread in susceptible varieties whereas no vine to vine disease spread seems to occur when the traditional native California sharpshooter vector species are transmitting the bacterium? The answer may be related to the feeding and inoculation locations of GWSS vs. other vectors. The GWSS will feed (and therefore inoculate vines) at the base of the canes, but the native vectors all feed almost exclusively at the tip of the cane. Inoculations at the tip of the cane probably require more time to move to an over-wintering refuge, so an early season inoculation is necessary for the infection to survive the winter and become chronic disease. Thus the window for vine to vine transmission leading to chronic disease would not exist. In this case only the early season primary spread from sources outside the vineyard would result in chronic disease, and because vine to vine transmission cannot begin until mid-season, these infections would be winter-cured.

If this hypothesis is correct, there are a number of possible consequences and conclusions that could improve PD management and control in areas where GWSS is present.

- The risk to growers of tolerant varieties is far less than has been previously assumed.
- There is a critical window of time somewhere in mid-season when susceptible vines need to be protected from vine to vine spread of PD. Chemical vineyard treatments early and late in the season that is before and after this window may be less effective than has previously been assumed.
- Economically important rates of secondary spread of PD may only happen in susceptible varieties and when large populations of GWSS are involved. Low but persistent populations of GWSS in Kern County do not appear to have resulted in appreciable losses from of vine to vine spread.
- Better targeted and timed chemical treatments could result in lower costs and be more compatible with other IPM programs.

- Late season vineyard surveys and rouging of infected vines is an important and cost effective management tool.
- The GWSS population treatment thresholds could be based on better epidemiological information, again possibly reducing overall PD management costs.

Because of the beneficial implications for PD management, it is important to experimentally test this hypothesis. We will be proposing to conduct experiments over the next two years to test the components of this hypothesis. The best experimental protocol would involve experiments conducted in two adjacent working vineyards, one tolerant and one susceptible variety. Ideally the experimental site would be in southern San Joaquin valley with climatological conditions representative of the viticulture areas of Kern or Tulare counties. It is critically important to everyone involved that these experiments do not create any new local PD problems or outbreaks. We have considered extensive safeguards in the design of these experiments. We intend for the risk to be very small, and the knowledge gained to be of great benefit in the practical control of PD in the southern San Joaquin and elsewhere in California. We would be happy to work collaboratively with other researchers and cooperators on various aspects of this research.

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FUNDING:

Funding for these projects was provided by the University of California, Division of Agriculture and Natural Resources, Pierce's Disease Research Grants Program and the Pierce's Disease/Glassy Wing.

Citrus Canker Threats to California Agriculture

Dan Opgenorth, Ph.D. Senior Plant Pathologist.

Citrus Bacterial Canker posed significant threats to the California Citrus industry in 2004. Shortly after the holiday season, gift packs of citrus fruit from Florida began to arrive in Northern California counties. Numerous oranges had lesions (Opgenorth, Fig. 1) resembling those caused by citrus canker. Since bacteria were found in association with the lesions, a presumptive diagnosis was made until additional work could be done to rule out the possibility of *Xanthomonas campestris* pv. c*itri* (*X.c.c*) [=*Xanthomonas axonopodis* pv. *citri*]. While a number of typical light yellow bacteria were isolated on tryptic soy agar (TSA), none of them tested positive when using the Agdia® indirect ELISA assay. Of greater significance was the interception of citrus bud wood from Japan by USDA inspectors in a box labeled as chocolates. The citrus canker pathogen was confirmed to be present in this plant material by USDA identifiers. When the destination address in Ventura County was investigated, a three acre plot of over three thousand grafted plants was found. Numerous samples were taken from the plot, as well as from the surrounding commercial citrus groves in the area, but the disease was not detected. Regulatory action was taken to destroy the illegal plants (Fig. 2), and the surrounding area is still being monitored for the citrus canker pathogen. If the citrus canker disease were ever to become established in California, it could seriously jeopardize the export of fresh citrus fruit, which is vital to the California Citrus Industry.

This scenario was predicted by Dr. Norman Schaad (USDA Foreign Disease Research Unit). Dr. Schaad had the foresight to work on the development of a rapid and accurate system of detection for this disease. Using an Exotic Pest Grant from the California Dept of Food and Agriculture, Dr. Schaad pioneered the use of Real Time PCR (Polymerase Chain Reaction) for the detection of *Xanthomonas campestris* pv. c*itri.* Since California does not have this disease, we tested the equipment and protocol at USDA interception points in California, specifically at the Port of San Francisco and at Los Angeles International Airport in early 2002 (1). The results were impressive and therefore the USDA ARS developed its own assay for use in an ongoing eradication action in Florida (2). At this time the Plant Pest Diagnostic Center has become part of a regional diagnostic laboratory network and received a Smart Cycler® in September of 2004 to use for our work. This report concerns some of the initial data generated in comparison of the two Real Time PCR Systems that can now be used for rapid detection of *Xanthomonas campestris* pv. c*itri*.

MATERIALS and METHODS

Our initial work with Real Time PCR was with the primers developed at the USDA APHIS PPQ by Vessela Mavrodieva (2). This assay uses a single primer set (MV 3/4) and the intercolating dye SYBR Green. The assay is currently used by the PPQ unit in Beltsville, Maryland as well as in Florida as a laboratory and field identification method. For comparison purposes, a Taq Man® assay using a primer set and specific fluorescent labeled probe developed by Dr. Schaad was used. This assay is being used in several Asian countries to test for the citrus canker pathogen on products that they export, and was previously used in California at the port facilities in 2002 (1).

To optimize both assays, a control was used at various dilutions. The control was a suspension of heat killed whole cells purchased from Agdia Inc. and previously used as a positive control in the indirect ELISA assay. Each Real Time PCR assay was used to test cultures taken from the various collections stored at CDFA. The cultures were initially grown on yeast-dextrose-carbonate (YDC) agar to test viability and purity. After purity was established, the cultures were

transferred to TSA agar, grown for 24 hours, then characterized using BIOLOG®. In this way, a group of closely related and suspect *Xanthomonas* plant pathogens was established for our testing purposes. The same inoculation fluid used for BIOLOG® testing was saved for use in both Real Time PCR assays. Since the concentration of cells could be important in detection of the bacteria, a dilution series from 0 to 1/10,000 was made using inoculation fluid for each culture. In the first series of tests, the MV primers were used with a master mix containing SYBR Green from Takara Inc®. Thus, the optimum concentration of cells for detection using Real Time PCR was established. Secondary testing used the MV primers with master mix containing SYBR Green from Applied Biosystems® and the Taq Man® primers and probe with a master mix from Applied Biosystems® without SYBR Green. The master mix from Applied Biosystems® required a hot start to activate the Taq Polymerase enzyme systems. Appropriate optimized conditions for cycling were used for each primer set and eight cultures were included in each run with positive and negative controls.

Results

The initial responses of 32 *Xanthomonas* isolates and one *Pseudomonas* (*Xanthomonas*-like) isolate when using the MV 3-4 primer set are given in Table 1. The average *X. c. c*. control was 18.18 Cycle threshold (Ct) units. Twelve of the other cultures had lower Ct values than the control, three were close to the control and eighteen were greater than the control. Those values that were less than the *X. c. c*. control and those that were not significantly different could easily be interpreted as positives for the Citrus Canker pathogen (59%). While a set of five consecutive 1/10 dilutions initially starting at approximately 10^{-7} was tested for each culture, only the dilution having the lowest Ct value was presented in this table. In Table 2, we can compare the Ct values for each culture when the optimum dilution was used with the MV primers and the Taq Man® primers with probe. With the MV 3-4 primers the average *X. c. c*. (1/100 dilution) control was 23.33 Ct units. Fourteen of the other cultures using the previously identified optimum dilutions had lower Ct values than the control, seven were close to the control and ten were greater than the control. That each of these dilutions produced a dramatic rise in the curve could easily allow one to consider that 67% of these cultures were potentially positive (Fig 3.). With the Taq Man® primers and probe the average X. c. c. (1/100 dilution) control was 27.13 Ct units. Eighteen of the other cultures produced a 0 Ct value and would be considered negative. Thirteen of the other cultures had a Ct value according to the standard of 5 SD units that was arbitrarily set (41%). However, none of these curves actually developed further and would be interpreted as flat, or negative for X. c. c. (Fig 4.).

Discussion;

In the consideration of Real Time PCR for the detection of citrus canker, the specificity of the assay should be of paramount importance. The initial work using *Xanthomonas* cultures from our collection demonstrated that both assays will identify *X. c. c*., however, the MV 3-4 primers will also give a response to a number of other *Xanthomonas* species. The Taq Man® primers and probe only produced a significant response to the single *X. c. c*. control that we were able to use. Thus, this assay could be more useful in initial determination of Citrus Canker as a diagnostic tool in our laboratory. Since many strains of the Canker pathogen are known and new strains continue to be discovered, a constant vigilance is necessary to insure that the specificity of the assay is still broad enough to allow all pathogenic strains to be identified. The MV 3-4 primers have been tested with all previously characterized and several novel Florida strains to date, and actually may have some utility in their breadth of response.

In order to further evaluate the utility of Real Time PCR for detection of citrus canker, epiphytic bacteria from suspect citrus samples should be evaluated to determine if false positives would be a problem. A collection of such epiphytes had been made by our laboratory over the past 12

years, but was lost this summer when a storage unit became unserviceable. Thus, we will continue to build a new collection of epiphytes from citrus and use them to evaluate our diagnostic techniques.

Of greatest utility would be the use of Real Time PCR to make rapid determinations directly from leaf or fruit samples without the initial time devoted to culture, growth and purification (usually several days to a week). This would allow samples to be rapidly screened upon arrival and limit further work to be conducted only on those specific lesions that produced a positive result. Thus, the task of obtaining a pure culture of the pathogen, which is necessary to make a significant regulatory diagnosis, would be greatly facilitated. Since the Smart Cycler® instrument and the PCR technology is hard-wired (and thus, has been designed for field use); a proposal to develop sampling methods and gain experience in the use of this technique for the direct evaluation of field samples will be immediately forthcoming.

ACKNOWLEDGEMENTS
We thank Dr. Douglas Prasher, formerly of USDA APHIS, who helped us initiate work with the SMART CYCLER and Rajinder Randhawa of CDFA who worked with the collection isolates and preformed the initial Real Time work.

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2 V. Mavrodieva, L. Levy and D.Gabriel Improved Sampling Methods for Real-Time Polymerase Chain Reaction Diagnosis of Citrus Canker from Field Samples. Journal of Bacteriology 94: 1 pp. 61-68.

Opgenorth, Figure 1. Orange with suspicious lesions.

Opgenorth Figure 2. Destruction of Citrus grove.

BIOLOG Identification	%	Source	Number	Dilution	MV 3-4 Ct
Xanthomonas					
Campestris					
Begonia A	97	CDFA	158	1/10	30.36
	93	CDFA	282	1/1000	35.26
Begonia B	99	UCBPP	876	1/10	12.89
	100	ICPPB	XP-207	0	14.15
campestris	100	ICPPB	XO-104	0	12.21
	96	ICPPB	XO-104	1/10	13.54
	87	ICPPB	XO-105	1/10	19.18
	99	ICPPB	XC-112	0	12.55
carotae	BF	UCBPP	875	0	14.26
	96	ICPPB	XV-219	0	14.8
dieffenbachiae	96	ICPPB	XT-126	1/10	35.38
	99	ICPPB	XC-174	1/10	32.36
juglandis	74	ICPPB	XJ-112	1/1000	34.49
malvacearum	BF	ICPPB	XP- 29	1/10	13.05
	100	ICPPB	XO-112	1/10	25.38
	98	ICPPB	XP-116	0	29.11
pelargonii	90	ICPPB	XP-166	1/10000	31.96
phaseoli	100	ICPPB	XP-172	0	14.78
	93	ICPPB	XP-21	1/10	18.62
	78	ICPPB	XP-20	1/10	18.62
poinsettiicola	90	ICPPB	XP-225	0	12.68
	100	ICPPB	XP 216	1/10	33.22
raphani	99	ICPPB	XC-122	0	30.07
	BF	ICPPB	XC-15	1/10	35.35
	BF	ICPPB	XC-13	1/100	34.30
	99	CDFA	265	1/100	36.46
vesicatoria	BF	Pepper	991	0	33.45
	97	ICPPB	XJ-7	1/10	29.03
	70	ICPPB	XP-110	1/100	32.69
oryzae oryzicola	BF	ICPPB	XO-110	0	11.85
	100	ICPPB	XO-111	1/10	14.53
	68	ICPPB	XJ-11	1/10	26.26
Pseudomonas					
cissicola	98	ICPPB	XF-118	1/1000	24.99
X. c. citri Control		Agdia		1/10	18.16*

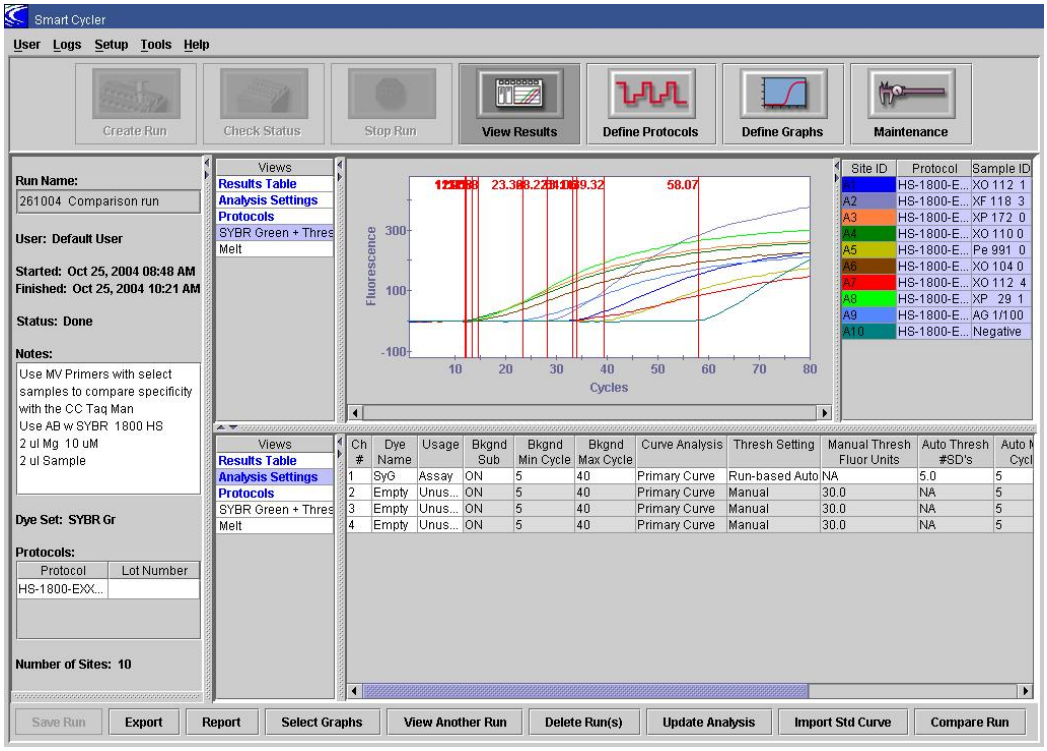
* This is an average of 33 experiments which range from 16.63 to 19.52

Opgenorth, Table 1. Real Time PCR response of known bacterial cultures when using MV 3-4 primers with SYBR Green as a fluorescent indicator.

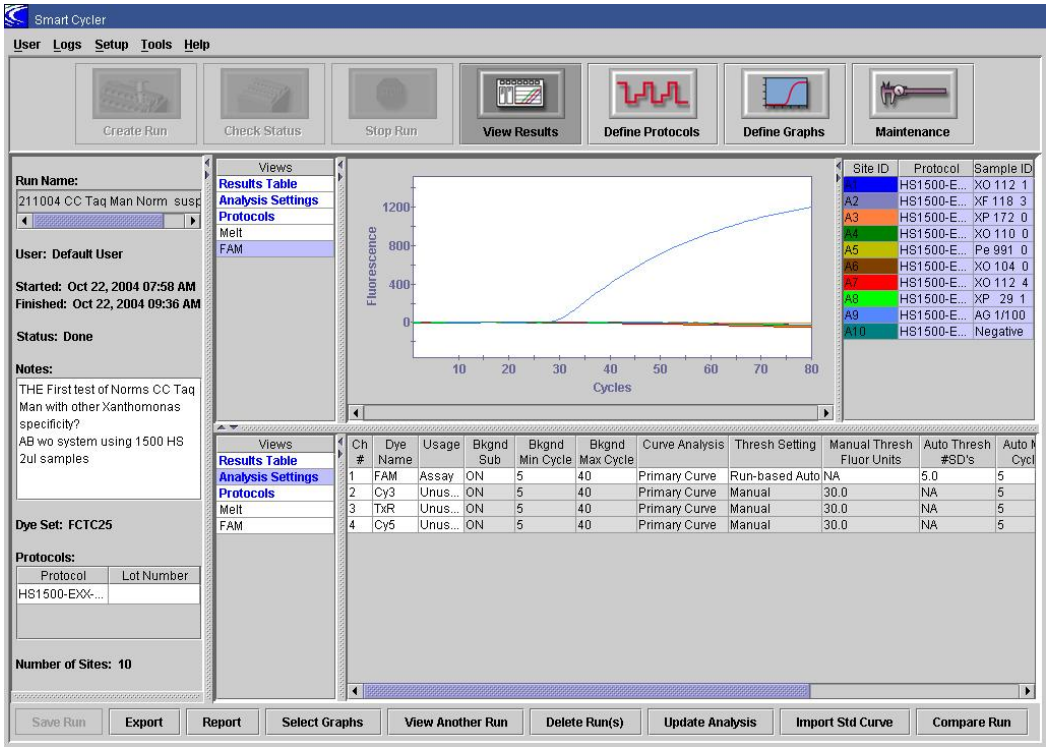
BIOLOG Identification	%	Source	Number	Dilution	MV 3-4 Ct*	Taq Man Ct
Xanthomonas						
campestris						
begonia A	93	CDFA	282	1/10	40.68	0
begonia B	99	UCBPP	876	1/10	12.82	30.00**
	100	ICPPB	XC-207	1/10	20.61	32.96**
campestris	100	ICPPB	XO-104	0	14.58	0
	87	ICPPB	XO-105	1/10	22.05	37.65**
	99	ICPPB	XC-112	1/10	19.16	37.66**
carotae	BF	UCBPP	875	0	16.71	39.64**
	96	ICPPB	XV-219	1/10	15.16	0
dieffenbachaie	96	ICPPB	XT-126	1/10	29.16	0
	99	ICPPB	XC-174	1/10	43.35	0
juglandis	74	ICPPB	XJ-112	1/10	45.39	39.91**
malvacearum	BF	ICPPB	XP-29	1/10	13.38	0
	100	ICPPB	XO-112	1/10	33.11	22.89**
	98	ICPPB	XP-116	0	32.03	0
pelargonii	90	ICPPB	XP-166	1/10000	37.35	50.00**
phaseoli	100	ICPPB	XP-172	0	11.95	0
	93	ICPPB	XP-21	1/10	14.08	0
	78	ICPPB	XP-20	1/10	19.64	0
poinsettiicola	90	ICPPB	XP-225	1/10	16.03	44.18**
	100	ICPPB	XP-216	1/10	54.63	47.24**
raphani	99	ICPPB	XC-122	0	28.19	0
	BF	ICPPB	XC-13	1/100	52.18	0
	BF	ICPPB	XC-15	1/10000	52.59	43.52**
	99	CDFA	265	1/10	49.85	0
vesicatoria	BF	Pepper	991	0	39.32	32.39**
	97	ICPPB	XJ-7	1/10	30.68	0
	70	ICPPB	XP-110	1/10	38.02	52.70**
oryzae						
oryzicola	BF	ICPPB	XO-110	0	12.21	0
	100	ICPPB	XO-111	1/10	15.92	0
	68	ICPPB	XT-11	1/10	27.38	0
Pseudomonas cissicola	98	ICPPB	XF-118	1/1000	28.22	0
Control 1/100 X.c.c. from Agdia Inc. (average of 4 runs)					23.33	27.13

**Ct acknowledged but no rise in the curve, essentially a 0 response. Probably due to the degree of sensitivity (standard deviation threshold) that we randomly set.
*All Ct values using the MV primers produce a noticeable rise in the curve, even those that are extremely late.

Opgenorth, Table 2.Comparison of Real Time PCR response of known bacterial cultures when using MV 3-4 primers with Applied Biosystems Master Mix with SYBR Green and Taq Man Norm Schaad primers and probe with Applied Biosystems Master Mix



Opgenorth Figure 3. Smart Cycler® data.



Opgenorth, Figure 4. Smart Cycler® data.

SEED HEALTH TESTING

Timothy Tidwell, Allen Noguchi, Diana Fogle,
YunPing Zhang, Jeanenne White, and Alex Ballesteros

Approximately three hundred seed health tests (Tidwell et al. Fig. 1) were performed in 2004. This involved testing for 35 different pathogens, in 22 different types of agricultural, horticultural, and tree seeds, representing 37 different seed clients.

Tidwell et al., Fig 1. Tomato seeds plated in agar for a seed health test.

The seed health laboratory staff also participated in a Seed Industry Conference in Woodland, by present ting a talk and practical laboratory display on the identification of sclerotia in seed samples. The presentation was geared towards professional registered seed technologists (RST) who perform seed germination and purity laboratory testing.

Contributions from Seed Health Testing staff were made to the National Seed Health System (NSHS) in 2004. Timothy Tidwell, a USDA-certified auditor for the NSHS in the Western United States performed audits of two California-based seed health testing laboratories. Consequently, a total of three private seed health testing laboratories in California are now accredited by the USDA and NSHS to conduct a number of key seed health tests, the results of which can be used as the basis for the USDA to write Phytosanitary Export Certificates. Additional audits of other seed health testing facilities are anticipated for 2005.

Wheat was again tested this past year for the Karnal Bunt (KB) Pathogen, *Tilletia indica* at the USDA laboratory facility in Blythe, CA. Although the total area of regulation was reduced to half of that in previous years (down to roughly 46,000 acres of wheat), the KB pathogen was still detected in five of eighty-nine fields in the Palo Verde Valley, near Blythe, California. Thus, the KB pathogen was confirmed to still be present in this area which is located in the southeastern California irrigated desert. A number of wheat seed

samples collected throughout California were also tested as part of the ongoing USDA national KB survey, but fortunately the pathogen appears to still be restricted to the very limited area of the Palo Verde Valley, near Blythe. In other KB developments, the PPDC was also identified by the USDA as a USA lab to test wheat seed which has been exported to other countries and determined to be infested with teliospores of *Tilletia indica* by scientists of the importing country. Our lab will be given the responsibility of confirming the presence of *T. indica* teliospores in samples of such seed shipments. Normally in the course of a year, the seed health testing laboratory also receives samples of wheat which is destined for export. These phytosanitary program samples are routinely tested for the Karnal Bunt pathogen, among other pathogens of phytosanitary concern prior to export.

MYCOLOGICAL PLANT PEST DIAGNOSTICS

Timothy Tidwell and Diana Fogle

Much of the time and energy of the mycology laboratory was diverted to the Sudden Oak Death (SOD) program in 2004. Yet, in addition to SOD work load and the routine sample diagnostics, a few new diseases popped up in California which found their way to our laboratory for diagnosis. *Peronospora radii*, a new downy mildew of Marguerite daisy, *Argyranthemum frutescens*, was detected in Coastal California—specifically in San Mateo, Santa Cruz, and Monterey Counties (Tidwell & Fogle, Figure 1). This marks the first appearance of this disease in North America. This disease became the subject of another cooperative study between researchers of the University of California Cooperative Extension (UCCE) and CDFA's Plant Pest Diagnostics. The pathogen seriously disfigures and stunts the growing point of the plants, which are commercially grown for use as potted ornamentals, cut flowers, and landscapes plants. The cooperative project resulted in a published Plant Disease Note (S.T. Koike, D. Fogle, S.A. Tjosvold, and A.I. King, 2004. Plant Disease 88:1163).

Tidwell & Fogle Fig. 1. *Peronospora radii*, a new downy mildew of Marguerite daisy, *Argyranthemum frutescens.* Note chlorosis of young foliage and grayish brown mycelium and sporangia (arrows) of the fungus. Photo by Steven Tjosvold.

Other new diseases detected in California in 2004 included a powdery mildew of *Romneya coulteri*, also known as the "Matilija poppy." The disease causes a typical disfiguring white mildew of the new foliage. The Matilija poppy (Tidwell & Fogle, Figure 2) is a native California shrub which is popular for use in xerophytic and native landscapes. The mildew pathogen is *Leveilula taurica*, which morphologically is the same species as the mildew which commonly attacks tomatoes and peppers, among other hosts. Preliminary inoculations using mildew spores from infected *Romneya* leaves to test their infectivity on tomatoes failed to confirm that it is, in fact, the same fungus that causes tomato mildew in California. Initially discovered independently, but at the same time, by Santa Barbara plant pathologist, Heather Scheck and Santa Cruz

Tidwell & Fogle, Figure 2. "Fried egg" flower of Matilija Poppy, *Romneya coulteri*. Photo by Michael Charter.

County Agricultural Biologist, Marilyn Perry, further study of this disease is planned for 2005.

In the "unusual sample" category, the paved roadways of a couple of Northern California counties were adversely affected by a fungus called *Pisolithus tinctorious*, also known as "PT," and the "dead man's foot" among other euphemistic nicknames. The fungus has a habit of making its presence known by pushing its fruiting body (a type of mushroom-like sporocarp) up along the sides of roads. In some cases, when asphalt has recently been laid down over the top of soil in which the fungus is established, the PT sporocarp simply pushes its way up (Tidwell & Fogle Fig. 3) through the new asphalt!

Tidwell & Fogle Figure 3. A very determined "PT " sporocarp pushing its way up through new asphalt. Photo courtesy of Glenn County Department of Agriculture.

Numerous samples of Chrysanthemum white rust, *Puccinia horiana* (Tidwell & Fogle Fig. 4) were submitted to the lab for diagnosis in 2004. Most of them originated from Santa Barbara County, but some also came from the South San Francisco Bay Peninsula Area of Northern California.

Tidwell & Fogle Fig. 4. Chrysanthemum White Rust on leaves. Note Buff-colored rust pustules (arrows).

PPDC mycology staff also participates in the National Plant Diagnostic Network, which is made up of experts from the nation's land grand universities and associated state departments and agencies. This network, of which CDFA serves together with UCD as the southwestern regional center for diagnostics, provides a cohesive system to quickly detect pests and pathogens that have been introduced into agricultural and natural ecosystems, identify them, and report them to appropriate responders and decision makers. In the Southeast United States, which saw multiple hurricanes in 2004, the primary soybean rust pathogen, *Phakopsora pachyrhizi*, was detected in no fewer than 9 southeastern states. Thus, California and our laboratory, in particular, will be on the alert for possible samples of beans, soybeans and other legumes with symptoms of rust for diagnosis this season

Another joint project with the CDFA Center for Analytical Chemistry was undertaken in 2004, this time involving research methods of testing alfalfa samples for the relative "moldiness" of alfalfa hay used for animal feed. In addition, a list identifying the molds of the alfalfa hay samples was produced.

Annual Survey of Stone Fruit and Grapevine Viruses for the Nursery Program

YunPing Zhang

California Fruit Tree, Nut Tee, and Grapevine Improvement Advisory Board (IAB) allocates funds annually to support the Nursery Registration and Certification (R&C) program. Under this program, deciduous fruit and nut trees and grapevines from participating nurseries are tested annually for various viruses to be used as a source of certified propagative materials in following years.

CDFA agriculture biologists coordinate with the nurseries and collect the samples. Samples collection is conducted at different growing seasons depending on the viruses to be tested. The nursery diagnostic laboratory tests all the samples collected from different nurseries mainly by Enzyme-Linked Immunosorbant Assay (ELISA), and Polymerase Chain Reaction (PCR) if necessary.

Deciduous fruit and nut tree viruses survey

Prune dwarf virus can cause many diseases such as sweet cherry blind wood and narrow leaf, sour cherry yellows, and Italian prune dwarf. Prunus necrotic ring spot virus can cause many diseases among stone fruit crops such as: cherry necrotic ring spot, cherry rugose mosaic, Prunus ring spot, stone fruit ring spot, sour cherry necrotic ring spot, tater leaf of peach and cherry, almond calico, and apricot line pattern. Both viruses are ilarviruses and very wide spread world wide and in California by means of transmission through grafting, seeds, and pollen.

To test for these viruses, 6-8 new shoots (1-2 inches long) are collected from the main scaffolds of the trees during early growing season of March through June. The samples are processed in the nursery diagnostic Laboratory and tested by ELISA using polyclonal antibodies for trapping the viruses and monoclonal antibody plus alkaline phosphatase conjugated goat anti-mouse antibody as probes in a combo ELISA test for both viruses.

In the year 2004, a total of 46,966 samples from 18 participating nurseries were tested for Prune dwarf virus and Prunus necrotic ring spot virus, of which 40,006 are R&C samples and 6,960 are service samples. A total of 498 (1.06%) samples were tested positive for PDV and/or PNRSV. Only 82 (0.2%) R&C samples while 416 (5.98%) service samples were tested positive for the two ilarviruses. Positive samples were further tested for each specific ilarvirus to determine their distribution. The result has revealed that 397 (79.7%) positive samples were infected with PNRSV while only 34 (6.8%) were infected with PDV and 67 (13.5%) were mixed infection by both viruses (Figure 1).

Grapevine viruses survey

Grapevine fan leaf virus is a nepovirus and nematode transmitted. It causes many types of symptoms including infectious malformation, yellow mosaic, and vein banding. Grapevine leaf roll disease is caused by several closteroviruses. So far, up to 9 types of closteroviruses have been associated with the disease. Some of them have been reported to be mealybug transmitted. Leaf roll virus 2 and 3 are most prominent in California and are surveyed by the program every year.

Young shoot samples are taking early in the season, around April for testing of GFLV while mature basal leaf are used late in the season, around October for leaf roll

virus diagnosis. All these viruses are tested in a double antibody sandwich F(ab)2 ELISA system.

For grapevine fan leaf virus, 1,329 composite samples from 6,645 grapevines were tested and no positives were detected. For grapevine leaf roll associated viruses 2 & 3, 1987 samples were tested and 47 were found to be infected by grapevine leaf roll associated virus 3. These tests have covered grapevines from 18 nurseries for fan leaf and 15 for leaf roll viruses.

The nursery annual survey program has played an important role in keeping California fruit and nut trees, and grapevines healthy. As shown in figure 2, for the past 5 years, the numbers of infected trees in the R&C program have been kept in a very low level while the numbers of infected trees not in the R&C program (service samples) were very high.

Figure 1. Percent of specific virus infection of positive samples

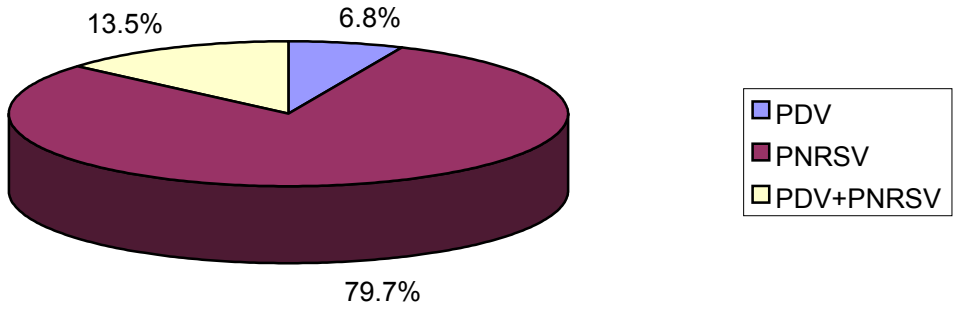
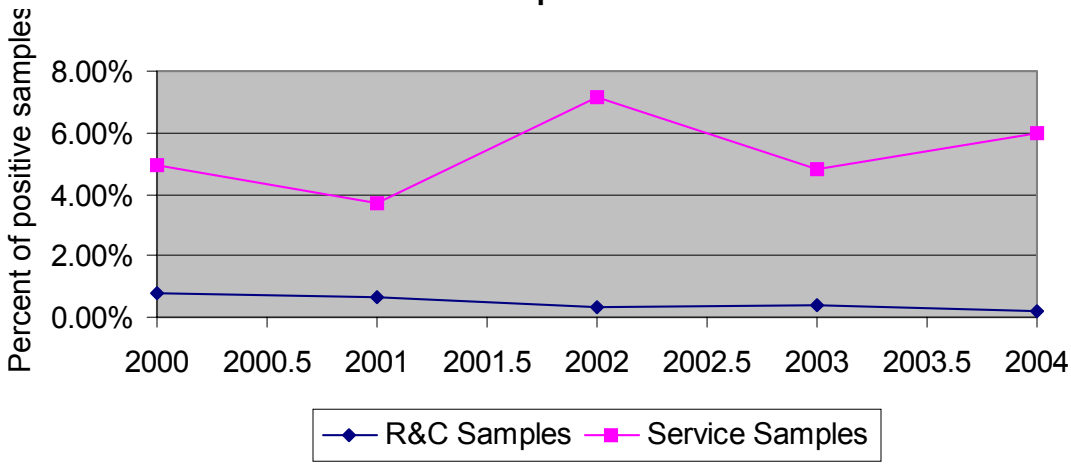


Figure 2. Detection of PDV/PNRSV from R&C and service samples



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Acknowledgements: This project is supported by California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board, Pest exclusion biologists, and participating nurseries.

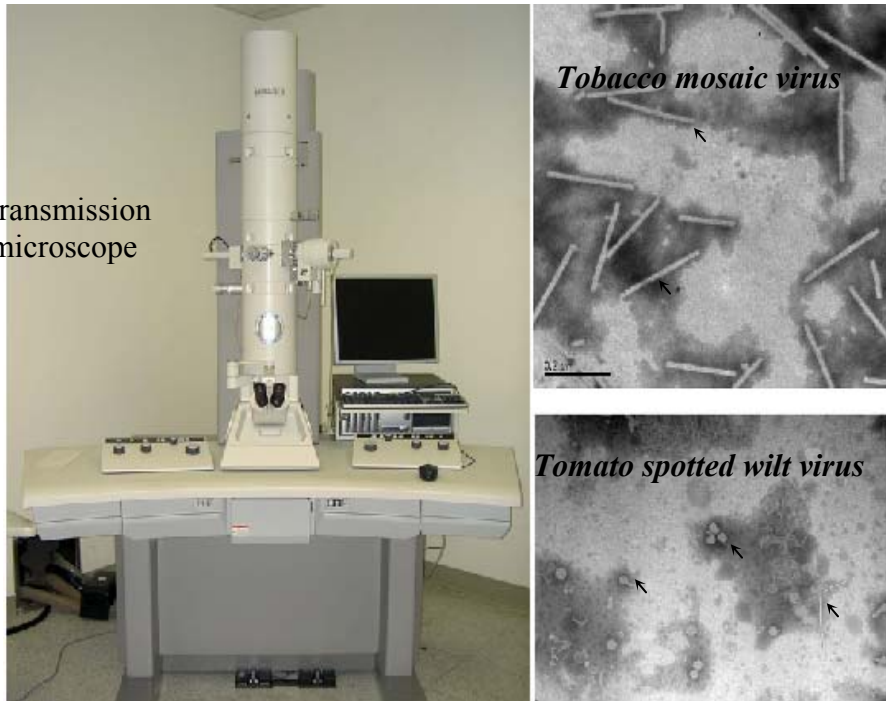
Annual Report from Plant Virology Laboratory-2004

Tongyan Tian, Ph. D., Senior Plant Pathologist (Diagnostician)
Plant Pest Diagnostics Center, CDFA

General: Plant Virology Laboratory is part of the Plant Pest Diagnostics Center responsible for plant virus disease diagnostics. We closely work with county plant pathologists and agriculture biologists, UC Extension and growers to diagnose various plant diseases caused by viruses. We analyze disease symptoms and determine proper test methods for further analysis. For routine samples, we often use serological methods, such as ELISA, for testing. When a serological method is not available or inconclusive, molecular-based detection methods are also used by the lab. Our Plant Virology Laboratory is capable of performing PRC and RT-PCR. In addition, transmission electron microscopy is also routinely used for confirmation of the presence of virus particles for plant samples.

In addition to the above routine plant virus detection, we also participate in other projects including the Plum Pox Virus statewide survey, and research to develop and improve virus identification methods for previously unknown viruses in the state.

H-7500 Transmission Electron microscope



Tian, Figure 1. Transmission electron microscopy is also routinely used for confirmation of the presence of virus particles. Arrows point to virus particles.

Plum pox virus disease survey for 2004:

In 2004, we continued the statewide survey for Plum Pox Virus (PPV). PPV causes severe disease (Sharka) on stone fruits. PPV was found in Pennsylvania in 1999. This virus disease has not been detected in California. This was our 5th year of PPV survey sponsored by U.S. Department of Agriculture. More than 7 Scientific Aids participated in

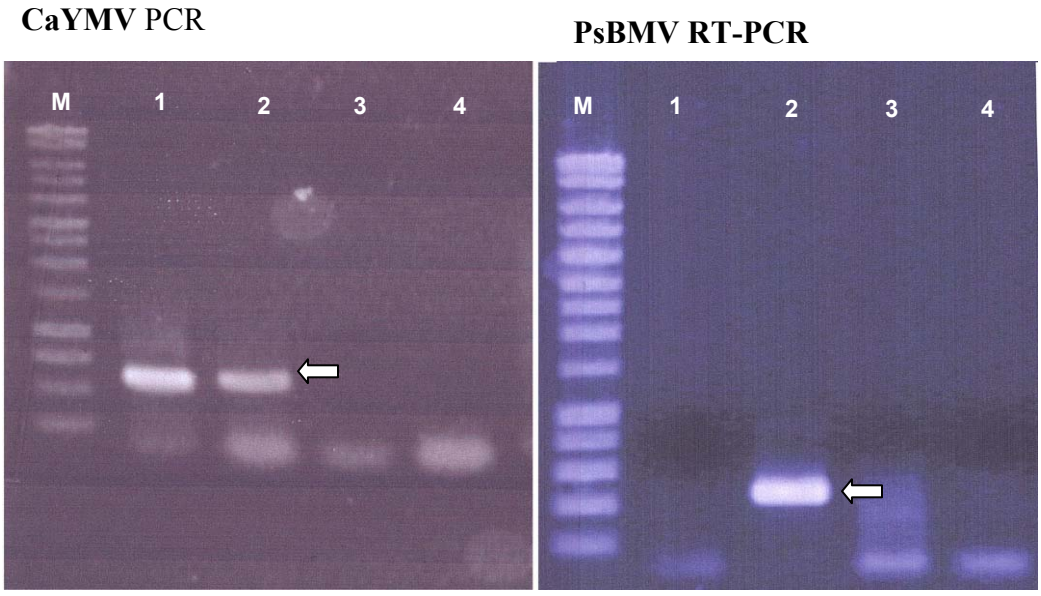
the program at the Plant Pest Diagnostics Center. Between April and June, we processed 29,465 samples using ELISA. All samples were negative in the ELISA test.

Viruses first detected in California:

1) Pea seed-borne mosaic virus (PsBMV):
Pea seed-borne mosaic virus is a member of potyvirus group. In 2004, we detected the presence of flexuous rod-shaped virus particles from several pea samples from Monterey County. Using PsBMV specific oligo-primers, we performed RT-PCR and nucleotide sequence analysis. Our results indicated that these pea plants were infected with PsBMV. PsBMV causes symptoms including leaf rolling, stunting, flower and seed pod malformation. The virus is spread by aphid vectors and is seed-borne. PsBMV is known to be in the U.S. However, this is the first time this virus was detected in the state.

2) Canna yellow mottle virus (CaYMV):

In December 2004, we received a canna sample from Santa Barbara County. The leaves showed typical symptoms of chlorosis and necrosis. We performed a PCR test using CaYMV specific oligo primers according to a recent publication by Momol *et al*. 2004. We were able to amplify a DNA product of approximately 560 bp. We were also able to purify bacilliform virus particles from the symptomatic plants. Based on these data, we concluded that these symptomatic canna plants were infected with CaYMV. CaYMV is a member of Badnavirus group and has been reported in two other states, Minnesota and Florida.



Tian Figure2. RT-PCR and PCR detection of CaYMV and PsBMV. Left panel: M = 1 KB DNA Marker; 1 = CaYMV infected canna; 2 = CaYMV infected canna; 3 = healthy canna; 4 = water control. Right panel: M = 1 KB DNA Marker; 2 = water control; 2 = PsBMV infected pea; 3 = healthy pea. Arrows point to the DNA products from virus infected plants.

Nematology Laboratory Staff:
John Chitambar
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A PCR method to identify eight common species of root knot nematodes (*Meloidogyne* spp.)

Ke Dong and John Chitambar

Nematology Laboratory
Plant Pest Diagnostics Center
California Department of Food and Agriculture

This report is a summary of currently available information on the application of DNA analysis to the species-level resolution in nematode diagnostics. Using a DNA analysis method several *Meloidogyne* species can be identified based on the infective second stage juveniles (J2s) alone. This is a very important feature as most field soil samples provided to the CDFA Nematology Laboratory contain J2s only. Here we report eight root-knot nematode species (*Meloidogyne* spp.) that can be tested, namely: *M. arenaria, M. chitwoodi, M. graminis, M. hapla., M. incognita, M. javanica, M. mayaguensis,* and *M. partityla*.

Meloidogyne chitwoodi, the Columbia root-knot nematode, is primarily of concern due to its effect upon potato tuber quality, and consequently the marketability of potatoes. The detection of *Meloidogyne chitwoodi* in a potato tuber can result in quarantine action against the shipment of seed or table stock potatoes destined for export, and thereby adversely affect the international trade market. Canada and Mexico currently will not accept seed potatoes from California grown in an area known to be infested with *M. chitwoodi* and require certification of potatoes free from *M. chitwoodi*. Mexico has two other species *M. hapla* and *M. javanica* also on the quarantine list. In addition, *M. partityla*, the pecan root-knot nematode with limited distribution in the USA, is on the USDA Western Region Nematode Survey list. A false positive identification could result in the quarantine of an entire production area, and a false negative could impact future trade relations. These regulatory important root-knot nematodes are required to be precisely identified to the species level by the CDFA Nematology Laboratory.

In the CDFA Nematology Laboratory, root-knot J2s are extracted from soil samples using a combined gravity sieve and misting method. Nematodes are examined using a dissection microscope at a magnification of 250X that allows preliminary assignment to genus. *Meloidogyne* J2s are further identified by light microscopy on temporary glass slides. A minimum of 20 infective juveniles of *Meloidogyne* is analyzed from each sample. An individual J2 is placed in a 15μl drop of 0.1M Tris-HCl (pH8.0) on a slide and crushed with a micropipette tip. The solution containing the crushed nematode is placed in individual PCR reaction tubes. A 5.0 μl portion of the solution serves as DNA template for PCR reaction. The PCR amplification is conducted with primer set C2F3/1108 (5' GGTCAATGTTCAGAAATTTGTGG 3' and 5'TACCTTTGACCAATCACGCT 3') located in the COII and 16S ribosomal mitochondrial genes respectively (1). PCR reaction master mix consists of 1.5 units of Taq Polymerase (Promega) in a 1x dilution of the 10x stock buffer, Mg^{+2} at 3.0mM final concentration, dNTPs each at 200μM final concentration, and each primer at 0.36μM final concentration. From the master mix, 25.0μl is aliquoted to a PCR tube containing 5.0μl nematode template and mixed thoroughly. Amplification conditions include an

initial denaturation at 94ºC for 2 minutes, followed by 45 cycles of denaturation at 94ºC for 1 minute, annealed at 50ºC for 1 minute, and extension at 72ºC for 2 minutes. A final extension step is conducted for 2 minutes at 72ºC.

The C2F3/1108 PCR amplification products (5.0μl of each mixed with 1.0μl loading buffer) are separated on a 1.0% agarose gel made with Agarose and 1.0x TAE buffer. The root-knot nematode species identifications are made by the size of amplification PCR (or PCR-RFLP) products (Table 1):

- The amplification product of 1.0kb is designated as *M. arenaria* (1)
- The amplification product of 705bp is *M. mayaguensis* (3)
- The amplification products of approximately 1.5kb are further digested with *Hinf*I.
    - If products of 1150bp and 350bp are produced the specimen is designated *M. incognita* (2)
    - If no digestion occurred the specimen is designated *M. javanica* (2)
- If the amplification products are about 520b-540bp. The PCR products are subjected to a *Dra*I digestion:
    - If the digestion products are 258bp, 119bp, 86bp, 40bp and 18bp, the species is *M. chitwoodi* (1).
    - If the digestion products are 307bp, 74bp, 42bp, 33bp, 32bp, 30bp, and 22bp, the species is *M. graminis* (4).
    - If the digestion products are 365bp, 78bp and 85bp, the species is *M. partityla* (5).
    - If the digestion products are 246bp, 198bp, 51bp and 33bp; or 444bp, 51bp and 33bp due to a single nucleotide mutant, the species is *M hapla* (1).

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Table 1. The root-knot species PCR products amplified from F2C3/1108 primers and digested with HinfI or DraI.

Species	Original size (PCR)	Digested size (PCR-RFLP)	Restriction enzyme
M. arenaria	1000bp	unnecessary	
M. incognita	1500bp	1150+350	HinfI
M. javanica	1500bp	1500 (not cut)	HinfI
M. mayaguensis	705bp	unnecessary	
M. chitwoodi	521bp	258+119+86+40+18	DraI
M. graminis	540bp	307+74+42+33+32+30+22	DraI
M. hapla	528bp	246+198+51+33	DraI
		(or) 444+51+33	DraI
M. partityla	528bp	365+85+78	DraI

2004 Annual Report of the Nematology Sample Processing Laboratory: Facts and Figures

John Chitambar, Ke Dong, Robert Hackney and René Luna

The Nematology Laboratory of the Plant Pest Diagnostics Branch comprises three Nematologists, one Agricultural Biological Technician and a support staff of two temporary employees.

Samples are routinely collected and sent to the Nematology Laboratory by County Agricultural and State personnel. These samples are designated to Quarantine, Nursery, Commercial, Dooryard (residential) or other zoological programs, and are sent as non-processed "raw" samples, or as processed samples of preserved nematode suspensions in vials. Approximately six counties have nematode sample processing facilities and personnel trained and certified by the State Nematology Laboratory. Plant parasitic nematodes are microscopic and inhabit above and below ground plant parts as well as rhizosphere soil of plants, depending on the species and biology of the nematode involved. Hence, samples comprised of plant and/or soil media are potentially inhabited by plant parasitic nematodes. The State Laboratory uses a combination of several scientific tests or procedures to extract nematodes from infested samples. Each of these procedures involves the use of large volumes of water, as nematodes are essentially aquatic animals requiring moisture for activity. The number of tests involved in extracting and preparing a collection of nematodes in clear water suspension for diagnostic evaluation is indication of the fact that the workload of the Nematology Sample Processing Laboratory cannot be entirely based on the number of samples processed.

During 2004 at total of 3,874 samples were diagnosed at the Laboratory. A breakdown of sample type per program is presented in Table 1. The bulk of quarantine samples include those entering the State through the External Quarantine for Burrowing and Reniform Nematodes program and those exported to other countries through the Quarantine Phytosanitary Certification Program. Samples in the former program comprise collections made mainly from indoor decorative foliage plants sold at nurseries, while samples in the latter program consists of mainly plant seeds processed and examined for targeted nematode species not wanted by importing countries. Most nursery samples of plants for sale by the grower comprised garlic (281 bulb samples), strawberries (828 foliage and root samples), grape and stone fruits (689 root and soil samples) collected through the State's Registration and Certification, and Nematode Control programs.

Chitambar, *et al.* Table 1. Total number of samples per program received by the CDFA Nematology Laboratory in 2004

Nematode Detection Program	No. of samples
Quarantine (*total*)	**2,030**
- Incoming External Quarantine	1,751
- Export Phytosanitary Certification	272
- Other	7
Nursery *(total)*	**1,814**
- Registration and Certification	1,125
- Nematode Control	689
Commercial	**13**
Dooryard/Residential	**0**
Other Zoological Identifications	**17**
Total	**3,874**

Table 2 shows the numbers of nematode samples submitted per county to the CDFA Nematology Laboratory. These numbers vary as they may be influenced by many factors among which include geographic location of county, number of nurseries per county, program and county laboratory facilities available.

Chitambar*, et al.* Table 2. Total number of samples submitted per County to CDFA Nematology Laboratory, 2004

County	No. of Samples	County	No. of Samples
San Joaquin	1,204	Sacramento	20
San Mateo	463	Los Angeles	19
Kern	320	Yolo	18
Merced	313	Kings	16
San Diego	264	Riverside	16
Madera	176	Colusa	15
Mono	103	Humboldt	6
Monterey	88	Santa Clara	6
Tehama	88	Sonoma	6
Shasta	85	Stanislaus	5
Solano	76	San Bernardino	4
Fresno	74	Butte	3
Lassen	72	Contra Costa	2
Siskiyou	57	Mendocino	2
Alameda	50	Orange	2
Imperial	47	El Dorado	1
Santa Cruz	41	Glenn	1
Lake	40	San Benito	1
San Luis Obispo	33	San Francisco	1
Sutter	21		

The Spiral nematode and Root Lesion nematodes were the most common species detected in all programs. While these two species groups are commonly found in California agricultural sites they can cause significant crop damage and loss and are therefore, of economic importance to local and international growers. Seven species of the Root Lesion Nematode were detected (Pratylenchus brachyurus, P. coffeae, P. penetrans, P. scribneri, P. thornei, P. vulnus and P. zeae). No A pests were detected in 2004.

The 36th and 37th Annual California Nematology Workshops

Robert W. Hackney

The CDFA Nematology Program (CDFANP) has co- sponsored 37 consecutive annual California Nematology Workshops along with the Departments of Nematology at the University of California, Davis and Riverside. During 2004 the 36th California Nematology Workshop was held at the University of California, Kearney Agricultural Center, Parlier, California. I convened the Steering Committee and laid the foundation (program planning, venue, invited speakers, etc.) for the 37th California Nematology Workshop in at the University of California, Davis on March 29, 2005. Since the California Nematology Workshop's recent funding through a grant from the University of California Division of Natural and Agricultural Resources (DANR) Nematology Work Group, a tradition has been established that at least one or more of the invited speakers will be selected from outside California and/or the United States. Participants frequently come from the international scientific community (i.e., outside the United States) as well as from California and other states. Continuing education credits are always available for the participants (i.e., pest control advisers and operators, growers and farmers, retail and wholesale nursery employees, arborists, landscapers, municipal and state employees, parks and recreation personnel, educators and consultants) who register to claim those units.

Complete details of the 36th and 37th California Nematology Workshop programs including speakers, their professional affiliations and their presentation titles/topics are archived on the CDFANP's [1]web site.

[1] http://www.cdfa.ca.gov/phpps/ppd/Nematology/NemaCaliWorkshop.htm
http://www.cdfa.ca.gov/phpps/ppd/Nematology/NemaCaliWorkshopArchive.htm

Announcing
36TH CALIFORNIA NEMATOLOGY WORKSHOP
March 30, 2004 – 8:00 a.m. to 4:30 p.m.
Kearney Agricultural Center
9240 S. Riverbend Ave. • Parlier, CA 93648
CONTACT PERSON: Lois Strole (559) 646-6545 Fax (559) 646-6593 Email lois@uckac.edu
Program Highlights
Morning Sessions
- Benefit of nematode protection during initial root development.
- Pre- and post-plant protection of peach trees without methyl bromide.
- Biological control of nematodes in vineyards.
- Coffee break with poster presentations encapsulating active projects statewide.
- Caenorhabditis elegans, how important it is and what is being done.
- Quarantine issues, Meloidogyne chitwoodi and rejection of potatoes moving into Mexico.

Lunch Provided

Afternoon Sessions
- Current legal requirements for applications of methyl bromide, Telone II and chloropicrin.
- Diagnosis of root lesion nematode species (new PCR methods).
- Are there variants of Mesocriconema xenoplax, ring nematode, across California?
- Familiarization with host plant-nematode databases.
- Movies on nematode feeding.
- Nematode morphology, identification and microscopy.
- Bacterial Canker Complex in the field.
- Methyl bromide alternatives.

PCA and Applicator Continuing Education Credit – pending • (Seating limited to the first 160 registrants)

Announcing

37th CALIFORNIA NEMATOLOGY WORKSHOP
March 29, 2005 – 7:30a.m. to 4:00 p.m.
Wellman and Hutchison Halls
UC Davis

CONTACT: Dr. Howard Ferris (530) 752.8432 Fax (530) 752.5809 Email hferris@ucdavis.edu
Dr. Robert Hackney (916) 262.1115 Fax (916) 262.1190 Email rhackney@cdfa.ca.gov

Morning Program
7:00-7:30 Poster Setup – Wellman Hall Lounge
7:30-8:15 Registration – Wellman Hall Lounge
8:15-8:30 Welcome and Program Overview – Wellman 2
8:30-9:15 Nemacur Registration Withdrawal: Challenges and Opportunities
Nematicide seed treatments
Nemacur replacements for turf
Nemacur replacements for grape
9:15-9:45 Nematicide Optimization Strategies

9:45-10:05 Break – Posters, Coffee and Juice – Wellman Hall Lounge

10:05-10:35 Understanding Host-plant Resistance
10:35-11:05 Department of Pesticide Regulation: Policy and Direction
11:05-12:00 New Faces and New Directions in Nematology
Presentations by – Graduate Students and Postdoctoral Fellows

Afternoon Program
1:15-1:30 Registration – Hutchison Hall
1:30-4:00 Four 30 minute rotations with 5 minute changeover
Rotation 1: Department of Nematology Laboratory Tour
Rotation 2: Sampling for nematodes, thresholds and food webs
Rotation 3: Ring Nematode Systematics, Diagnostics, Damage and Management
Rotation 4: Nematode and Soil Biological Diversity

7 hours PCA credit pending (includes laws and regulations hours)

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	*Adelges (*Sacchiphantes) *abietis* (submitted 2004)
Aphididae	*Cinara cupressi* complex (2004)
Diaspididae	*Aonidomytilus albus* (submitted 2004)
	Chrysomphalus dictyospermi (submitted 2004)
Eriococcidae	*Cryptophagus fagisuga* (2004)
Margarodidae	*Icerya purchasi* (revision 2004)
Pseudococcidae	*Maconellicoccus hirsutus* (1998, revised 2004)
	Phenacoccus manihoti (revised 2004)

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Case 2: The description of a new mealybug species enables implementation of a successful biological control programme across Africa, saving billions of US$.

Case 9: Lack of taxonomic expertise results in extended loss of coffee crops.

Case 10: Correct identification of pest prevents mango crop destruction and saves millions.

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